U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-251 828

WASTEWATER LABORATORY PROCEDURES AND CHEMISTRY

Environmental Protection Agency

June 1975

TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA 907/9-75-002	2.	3. RECIPIENT'S ACCESSION NO.
4. TITLE AND SUBTITLE Wastewater Laboratory Procedures and Chemistry		5. REPORT DATE June 1975
		6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS U. S. Environmental Protection Agency Office of Intermedia Programs Manpower and Training Program Region VII, 1735 Baltimore, Kansas City, Missouri 64108		10. PROGRAM ELEMENT NO.
		11. CONTRACT/GRANT NO. 5TT1-WP-16-03
12. SPONSORING AGENCY NAME AND ADDRESS Same as Box 9		13. TYPE OF REPORT AND PERIOD COVERED
		14. SPONSORING AGENCY CODE

15. SUPPLEMENTARY NOTES

16. ABSTRACT

This manual has been adapted from Chapter 14 of "Operation of Wastewater Treatment Plants - A Field Study Course" for limited distribution in Region VII. This manual is intended to serve as a training reference material for personnel of the Regional Surveillance and Analysis Program to assist them in providing assistance to treatment plant operators who have been identified as being in need of greater skills to perform necessary laboratory analyses.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Water Pollution Control Laboratory Analysis	Water Pollution Control	

PRICES SUBJECT TO CHANGE

18. DISTRIBUTION STATEMENT Unlimited	19. SECURITY CLASS (This Report) UNCLASSIFIED	21. NO. OF PAGES 218
	20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE $7.75

EPA Form 2220-1 (9-73)

114095

PB 251 828

WASTEWATER LABORATORY PROCEDURES & CHEMISTRY

U.S. ENVIRONMENTAL PROTECTION AGENCY

REGION VII

1735 BALTIMORE
KANSAS CITY, MISSOURI - 64108

OFFICE OF INTERMEDIA PROGRAMS
MANPOWER & TRAINING PROGRAM

SURVEILLANCE & ANALYSIS DIVISION
TECHNICAL SERVICES BRANCH

JUNE 1975

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U. S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

EPA 907/9-75-002

WASTEWATER

LABORATORY PROCEDURES AND CHEMISTRY

This manual has been adapted from Chapter 14 (by James Patterson) of "Operation of Wastewater Treatment Plants - A Field Study Course."

The complete Field Study Course has been prepared for EPA by California State University - Sacramento and is available at a nominal charge. For information on ordering the complete course, write to:

Dr. Kenneth D. Kerri
Department of Civil Engineering
California State University
6000 Jay Street
Sacramento, California 95819

Environmental Protection Agency
Region VII
1735 Baltimore
Kansas City, Missouri 64108

Office of Intermedia Programs
Manpower & Training Program

Surveillance & Analysis Division
Technical Services Branch

JUNE 1975

EPA REVIEW NOTICE

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OBJECTIVES

Chapter 14. Laboratory Procedures and Chemistry

Following completion of Chapter 14 you should be able to:

1. Work safely in a laboratory.
2. Know how to operate laboratory equipment.
3. Collect representative samples of influents to and effluents from a treatment process as well as sample the process.
4. Prepare samples for analysis.
5. Perform plant control tests.
6. Recognize shortcomings or precautions for the plant control tests.
7. Record laboratory results.

CHAPTER 14. LABORATORY PROCEDURES AND CHEMISTRY

14.0 INTRODUCTION

14.00 Should You Start This Lesson Now?

Laboratory procedures and results are the means by which we control the efficiency of our treatment processes and measure the effectiveness of the processes. To operate your plant as efficiently as possible, you must understand the laboratory procedures and relate them to the actual operation of your plant.

This lesson has been given to you at this time mainly for reference purposes. When you read the lessons on the treatment processes you should begin to wonder how certain tests are performed that are essential for proper plant operation. At this time you should refer to this lesson for a general discussion and a description of the laboratory procedure.

It might seem logical to you to complete this lesson first in order to better understand the operational aspects of the treatment process lessons. Many operators and potential operators who were interested in this profession have taken this course. Most of them have said that they wanted to learn about the treatment processes first and then learn how to apply the lab procedures to plant operation. Many potential operators experienced difficulty with the terminology when they tried to work this chapter before completing the lessons on the treatment processes. If you are an experienced operator and are anxious to learn more chemistry and to obtain a better understanding of lab procedures, you may decide to try this lesson first.

Past experience has indicated that most operators prefer to use this section as a reference while studying the lessons on treatment processes. You are the operator who wants to learn more about treatment plant operation, and you are encouraged to use this material in any manner that you feel best fits your particular situation and professional goals. Now is the time for you to decide whether you are going to:

1. Thumb through this lesson, proceed through the chapters on treatment processes, and then complete this lesson;
2. Complete the lessons on treatment processes, referring to this lesson when interested, and then complete this lesson;
3. Complete this lesson and then the lessons on treatment processes; or
4. Follow your own plan.

14.01 Material in This Lesson

A few of the lab procedures outlined in this chapter are not "Standard Methods" (4),[1] but are used by many operators because they are simple and easy to perform. Some of these procedures are not accurate enough for scientific investigations, but are satisfactory for successful plant control and operation. When lab data must be submitted to regulatory agencies for monitoring and enforcement purposes, you should request the agency to provide you with a list of approved test procedures.

Each test section contains the following information:

1. Discussion of test.
2. What is tested?
3. Apparatus.
4. Reagents.
5. Procedures.
6. Precautions.
7. Examples.
8. Calculations.

---

[1] Numbers in parentheses refer to references in Section 14.02.

If you would like to read an introductory discussion on laboratory equipment and analysis, the Water Pollution Control Federation has a good publication entitled "Simplified Laboratory Procedures" (3). Good discussions on the use of the analytical balance may be found in "Laboratory Procedures" (1) or "Simplified Procedures" (3).

14.02 References

1. "Laboratory Procedures for Operators of Water Pollution Control Plants" by Joe Nagano. Obtain from Secretary-Treasurer, California Water Pollution Control Association, P.O. Box 61, Lemon Grove, California 92045. Price $3.25 to members of CWPCA; $4.25 to others.

2. "EPA Methods for Chemical Analysis of Water and Wastes", Analytical Quality Control Laboratory, 1014 Broadway, Cincinnati, Ohio 45202 (October 1974)

3. "Simplified Laboratory Procedures for Wastewater Examination," WPCF Publication No. 18, 1968, 60 pages. $2 to WPCF members; $3 to others.

4. "Standard Methods for Examination of Water and Wastewater," 13th Edition, 1971, 874 pages. $16.50 to WPCF members; $22.50 plus postage to others.

Both References 3 and 4 may be obtained by writing:

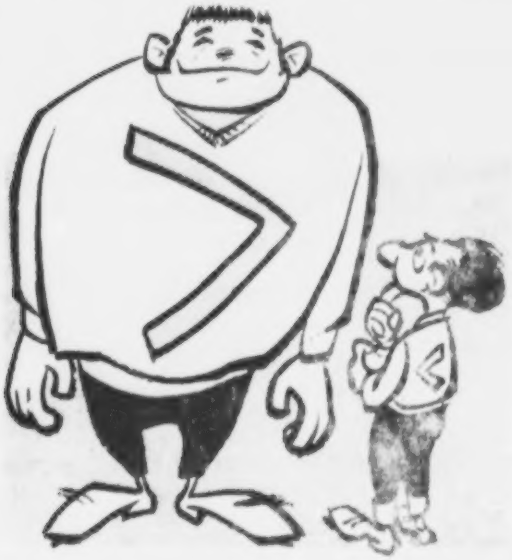
Water Pollution Control Federation
3900 Wisconsin Avenue
Washington, D.C. 20016

Order forms may be found in the Journal of the Water Pollution Control Federation.

14.03 Acknowledgments

Many of the illustrated laboratory procedures were provided by Mr. Joe Nagano, Laboratory Director, Hyperion Treatment Plant, City of Los Angeles, California. These procedures originally appeared in Laboratory Procedures for Operators of Water Pollution Control Plants, prepared by Mr. Nagano and published by the California Water Pollution Control Association. The lists of equipment, reagents, and procedures outlined in this chapter are similar to those listed in the references in Section 14.02. Use of information from these references is gratefully acknowledged.

14.1 GLOSSARY OF TERMS AND EQUIPMENT



14.10 Terminology

> Greater than.

DO > 5 mg/1, would be read as DO greater than 5 mg/1.

< Less than.

DO < 5 mg/1, would be read as DO less than 5 mg/1.

Aliquot (AL-li-kwot). Portion of a sample.

Ambient Temperature (AM-bee-ent). Temperature of the surroundings.

Amperometric (am-PURR-o-MET-rick). A method of measurement that records electric current flowing or generated, rather than recording voltage. Amperometric titration is an electrometric means of measuring concentrations of substances in water.

Anaerobic Environment (AN-air-O-bick). A condition in which "free" or dissolved oxygen is not present.

Blank. A bottle containing dilution water or distilled water, but the sample being tested is not added. Identical tests are frequently run on a sample and a blank and the differences compared.

Buffer. A measure of the ability or capacity of a solution or liquid to neutralize acids or bases. This is a measure of the capacity of water or wastewater for offering a resistance to changes in the pH.

Composite (proportional) Samples (com-POZ-it). Samples collected at regular intervals in proportion to the existing flow and then combined to form a sample representative of the entire period of flow over a given period of time.

Distillate. In the distillation of a sample, a portion is evaporated; the part that is condensed afterwards is the distillate.

End Point. Samples are titrated to the end point. This means that a chemical is added, drop by drop, to a sample until a certain color change (blue to clear, for example) occurs which is called the end point of the titration. In addition to a color change, an end point may be reached by the formation of a precipitate or the reaching of a specified pH. An end point may be detected by the use of an electronic device such as a pH meter.

Flame Polished. Sharp or broken edges of glass (such as the end of a glass tube) are flame polished by placing the edge in a flame and rotating it. By allowing the edge to melt slightly, it will become smooth.

M or Molar. A molar solution consists of one gram molecular weight of a compound dissolved in enough water to make one liter of solution. A gram molecular weight is the molecular weight of a compound in grams. For example, the molecular weight of sulfuric acid (H_2SO_4) is 98. A 1M solution of sulfuric acid would consist of 98 grams of H_2SO_4 dissolved in enough distilled water to make one liter of solution.

Molecular Weight. The molecular weight of a compound in grams is the sum of the atomic weights of the elements in the compound. The molecular weight of sulfuric acid (H_2SO_4) in grams is 98.

Element	Atomic Weight	Number of Atoms	Molecular Weight
H	1	2	2
S	32	1	32
O	16	4	64
			98

N or Normal. A normal solution contains one gram equivalent weight of a reactant (compound) per liter of solution. The equivalent weight of an acid is that weight of a compound which contains one gram atom of ionizable hydrogen or its chemical equivalent. For example, the equivalent weight of sulfuric acid (H_2SO_4) is 49 (98 divided by 2 because there are two replaceable hydrogen ions). A 1N solution of sulfuric acid would consist of 49 grams of H_2SO_4 dissolved in enough water to make one liter.

Oxidation (ox-i-DAY-shun). Oxidation is the addition of oxygen, removal of hydrogen, or the removal of electrons from an element or compound. In wastewater treatment, organic matter is oxidized to more stable substances.

Percent Saturation. Liquids can contain in solution limited amounts of compounds and elements. 100% saturation is the maximum theoretical amount that can be dissolved in the solution. If more than the maximum theoretical amount is present, the solution is supersaturated.

$$\% \text{ Saturation} = \frac{\text{Amount in Solution}}{\text{Maximum Theoretical Amount in Solution}} \times 100\%$$

Reagent (re-A-gent). A substance which takes part in a chemical reaction that is used to measure, detect, or examine other substances.

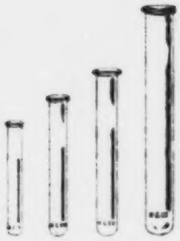
Representative Sample. A portion of material or water identical in content to that in the larger body of material or water being sampled.

Titrate. To titrate a sample, a chemical solution of known strength is added on a drop-by-drop basis until a color change, precipitate, or pH in the sample is observed (end point). Titration is the process of adding the chemical solution to completion of the reaction as signaled by the end point.

14.11 Equipment

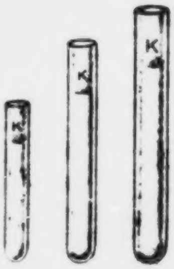
Equipment can be better described by a photo or a sketch than a written description; consequently, this portion of the glossary will describe equipment in this manner. Photos of equipment shown were provided by Van Waters & Rogers.

ILLUSTRATIONS OF LABORATORY APPARATUS



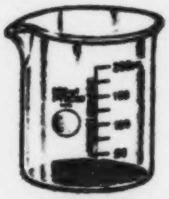
60809-021 Series

Test Tube



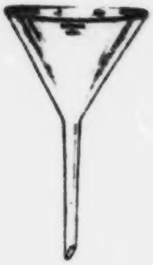
60824-116 Series

Culture Tube
Without Lip



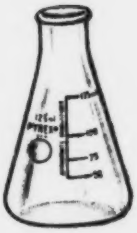
13912-287

Beaker



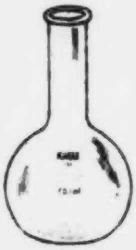
30200-025

Funnel



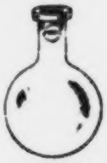
29140-023

Flask,
Erlenmeyer
(ER-len-MY-er)
Wide Mouth



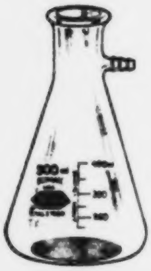
29110-102

Flask,
Boiling
Flat Bottom



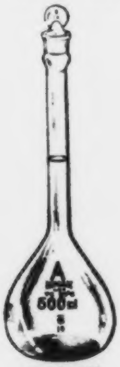
29126-022

Flask,
Boiling
Round Bottom
Short Neck



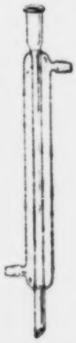
29415-100

Flask,
Filtering



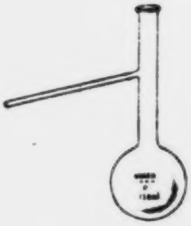
29619-842

Flask,
Volumetric



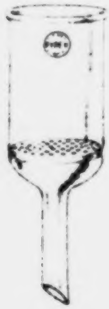
23130-049
23131-020

Condenser



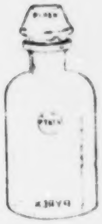
29209-083

Flask,
Distilling



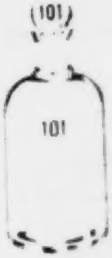
30294-024

Funnel,
Buchner
With
Perforated Plate



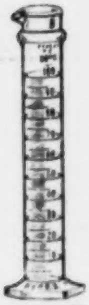
16269-027

Bottle,
Reagent



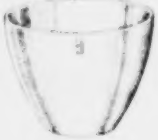
16285-114

Bottle,
BOD



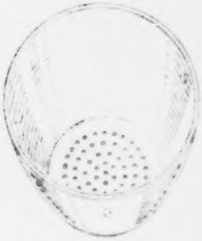
24707-265

Cylinder,
Graduated



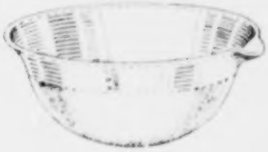
23810-021

Crucible
Porcelain



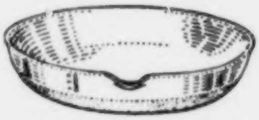
23835-000

Crucible
Gooch
(GOO-ch)
Porcelain



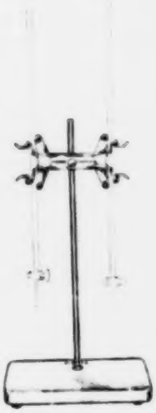
25310-019

Dish,
Evaporating



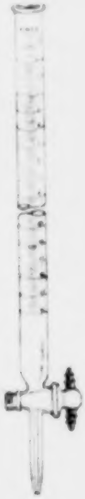
25313-017

Dish,
Evaporating
Shallow Form



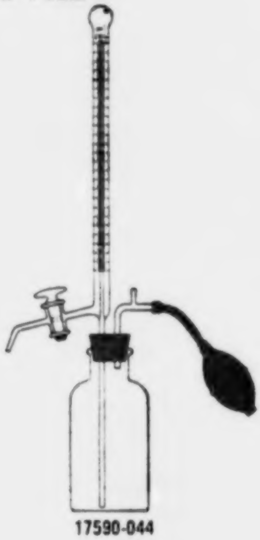
17685-005

Support, Buret
& Buret Clamp



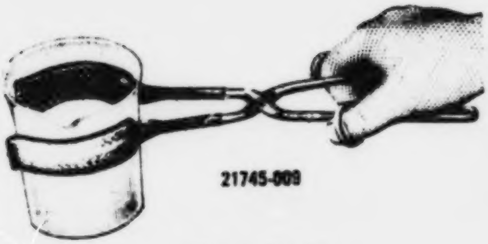
17454-443

Buret
(bur-RET)



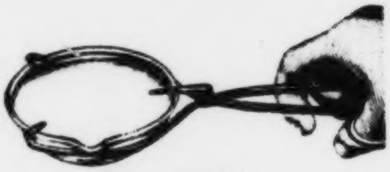
17590-044

Buret
Automatic



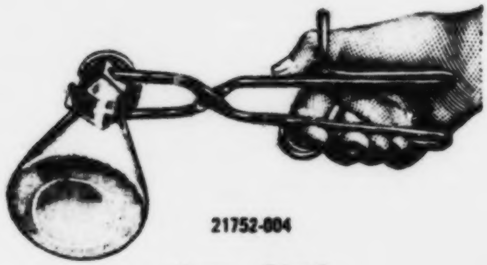
21745-009

Clamp, Beaker,
Safety Tongs



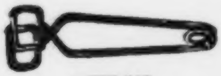
21750-009

Clamp, Dish,
Safety Tongs



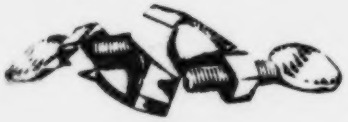
21752-004

Clamp, Flask,
Safety Tongs



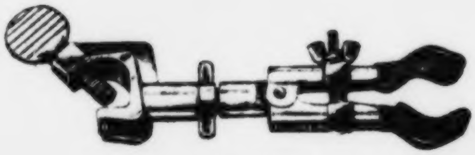
21770-029

Clamp, Test Tube



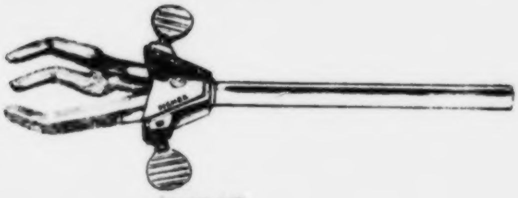
21677-000

Clamp Holder



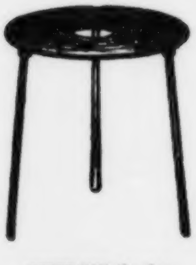
21611-046

Clamp, Utility



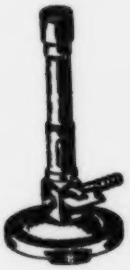
21633-049

Clamp



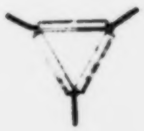
62765-029 Series

Tripod, Concentric
Ring



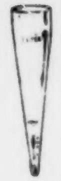
17951-029

Burner, Bunsen

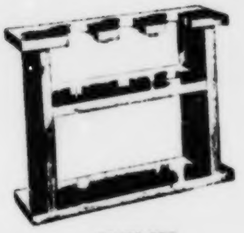


62730-024 Series

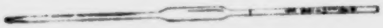
Triangle
Fused



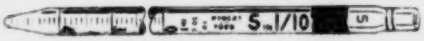
66187-004
Cone,
Imhoff
(IM-hoff)



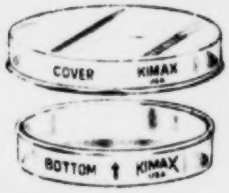
66190-009
Cone Support



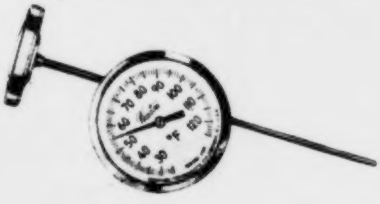
53047-024 Series
Pipette
(PIE-pet)
Volumetric



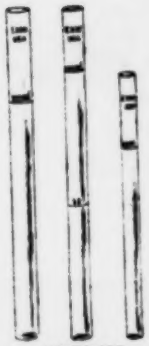
53224-028 Series
Pipet, Serological



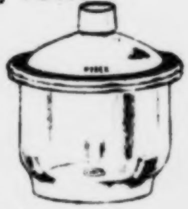
25353-248
Dish, Petri



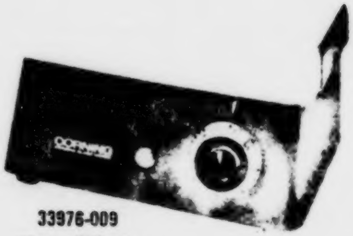
61048-033 Series
Thermometer, Dial



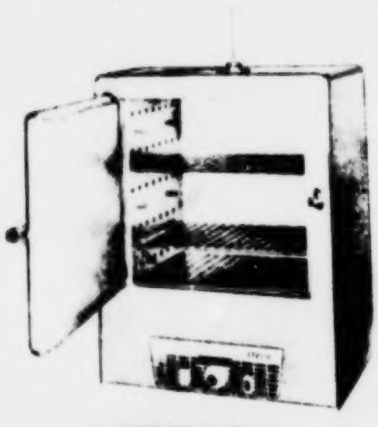
66176-325
Color Comparison
Tubes, Nessler



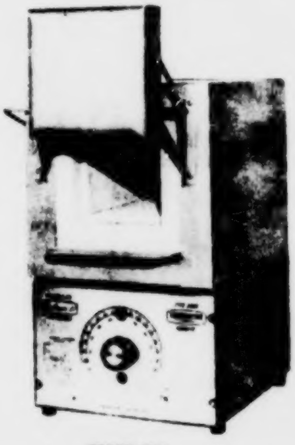
25026-026
Desiccator
(DES-ick-kay-tor)



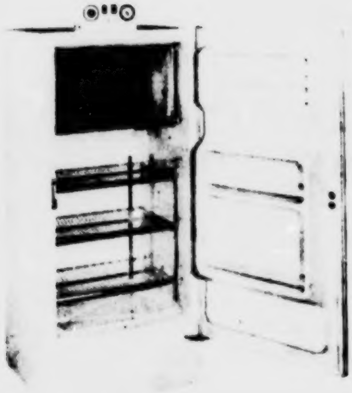
33976-009
Hot Plate



52368-022 Series
Oven, Mechanical Convection

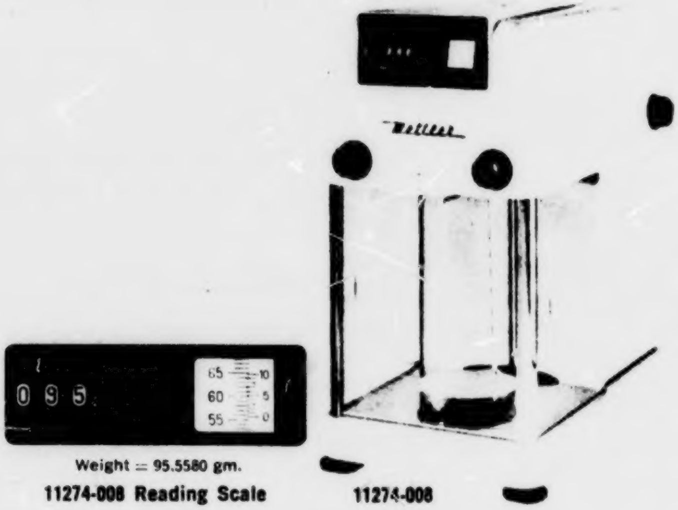


30632-003
Muffle Furnace, Electric



35960-000

BOD Cabinet


0 9 5
65
60
55
10
5
0
Mettler

Weight = 95.5580 gm.

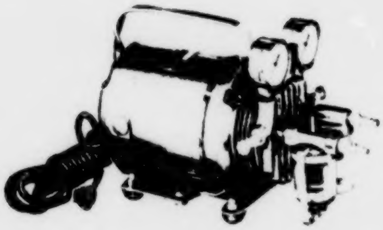
11274-008 Reading Scale

11274-008

Balance, Analytical

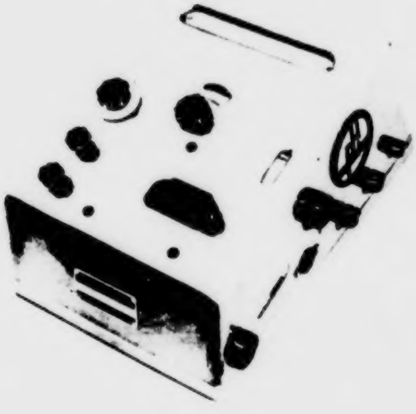
34114-055

pH Meter



54906-001

Pump, Air Pressure & Vacuum



57980-000

Spectrophotometer

pHydrion Papers
B
DOUBLE ROLL
pH1 3 5 7 9 11

60776-002

Test Paper, pH 1-11

14.2 SAFETY AND HYGIENE, by A.E. Greenberg from California Water Pollution Control Association Operators Laboratory Manual

14.20 Laboratory Safety

Safety is important in the laboratory as well as in the rest of the treatment plant. Therefore, each employee working in a laboratory should be thoroughly familiar with this section.

On questions of safety, consult your state's General Industrial Safety Orders or similar document and Sax's "Dangerous Chemicals".[2]

Personnel working in a wastewater treatment plant laboratory must realize that a number of hazardous materials and conditions exist. PREVENT ACCIDENTS. Be alert and careful. Be aware of potential dangers at all times. The major threats to you are listed for your safety.

1. Infectious Materials

   Wastewater and sludge contain millions of bacteria, some of which are infectious and dangerous, and can cause diseases such as tetanus, typhoid, dysentery, poliomelytis, and hepatitis. Personnel handling these materials should thoroughly wash their hands with soap and water, particularly before handling food. Do not pipette wastewater or polluted samples by mouth. Use a rubber bulb. Though not mandatory, inoculations by your County Health Department are recommended for each employee.

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[2] See Sax, N.I., Dangerous Properties of Industrial Materials, Third Edition, Reinhold, New York, 1968, price $35.

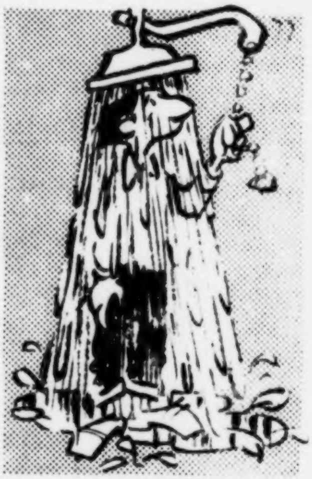
2. Corrosive Chemicals

A. Acids

(1) Examples: Sulfuric, hydrochloric, nitric, glacial acetic, Pomeroy solutions Nos. 1 and 2, and chromic acid cleaning solutions.

(2) Acids are extremely corrosive to human tissue, metals, clothing, wood, cement, stone, and concrete. Use glassware or polyethylene containers.

(3) In case of accidental spills, immediately dilute with large portions of water and neutralize the acid with sodium carbonate or bicarbonate until bubbling and foaming stops. Clean up neutralized material. If spills occur on bench tops, dilute, neutralize, and squeegee into sink. If spills occur on person, immediately wash off with water. If spills occur on face (spills of concentrated acid), immediately flood with large quantities of cold water. Notify supervisor. Remember to add acid to water, but not reverse. Pour and pipette carefully to prevent spilling and dropping. Prevent contact with metals, particularly equipment.



B. Bases

(1) Examples: Sodium hydroxide, potassium hydroxide, ammonium hydroxide, alkaline iodide---sodium azide solution.

(2) Handle with extra care and respect. They are extremely corrosive to skin, clothing, and leather. Use glassware and polyethylene containers.

Ammonium hydroxide is extremely irritating to the eyes and respiratory system. Pour ammonium hydroxide under a laboratory hood with fan in operation.

(3) In case of accident, wash with large quantities of water and use saturated boric acid solution to neutralize.

C. Miscellaneous

(1) Chlorine gas solution---avoid inhalation. Handle in hood. Secure cover to prevent escape of vapors.

(2) Ferric salts, Ferric chloride---very corrosive to metals. Avoid body contact and wash off immediately.

(3) Strong oxidants---avoid body contact. Wash off immediately. Use of perchloric acid by untrained personnel must be prohibited.

3. Toxic Materials

Avoid ingesting or inhaling.

A. Solids: Cyanides, chromium, cadmium, and other heavy metal compounds.

B. Liquids: Use in vented hood. Carbon tetrachloride, ammonium hydroxide, nitric acid, bromine, chlorine water, aniline dyes, formaldehyde, chloroform, and carbon disulfide. Carbon tetrachloride is absorbed into skin on contact; its vapors will damage the lungs; and it will build up in your body to a dangerous level.

C. Gases: Use in vented hood. Hydrogen sulfide, chlorine, ammonia, nitric, hydrochloric acid.

D. Most laboratory chemicals have toxicity warnings and antidotes on their labels. Learn about the materials you use. Don't breathe, eat, or drink them; and if they come in contact with your body, quickly apply large quantities of water to wash the substance away.

4. Explosive or Inflammable Materials

   A. Gases: Acetylene, hydrogen.

   B. Liquids: Carbon disulfide, benzene, ethyl ether, petroleum ether, acetone, gasoline.

   Store these materials according to fire regulations to prevent fire hazards. If large quantities must be stored, they should be located in a separate storage building.

   Do not use near open flame or exposed heating elements. Use under a vented laboratory hood. Do not distill to dryness or explosive mixtures may result. Use face mask. Do not throw flammable liquids into sinks. Cigarette discard may cause fire. Do not let gas cylinders fall.

5. Broken Equipment

   A. Inexpensive Items--Beakers and flasks should be discarded, except for minor chips which can be flame polished[3] easily.

   B. Expensive Items--Should be set aside for salvage if possible. Discard if damaged beyond repair.

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[3] Flame Polished. Sharp or broken edges of glass (such as the end of a glass tube) are flame polished by placing the edge in a flame and rotating it. By allowing the edge to melt slightly, it will become smooth.

6. Miscellaneous

A. Use safety goggles or face mask in any experiment in which there is danger to the eyes. Never look into the end of the test tube during reaction or heating.

Use care in making rubber-to-glass connections. Lengths of glass tubing should be supported while they are being inserted into rubber. The ends of the glass should be flame polished, and either wetted or covered with a lubricating jelly for ease in joining connections. Never use grease or oil. Gloves or grippers should be worn when making such connections, and the tubing should be held as close to the end being inserted as possible to prevent bending or breaking.

Never try to force rubber tubing or stoppers from glassware. Cut the rubber or material off.

B. Always check labels on bottles to make sure that the chemical selected is correct. All chemicals and bottles should be clearly labeled. Never handle chemicals with bare hands. Use spatula, spoon, or tongs.

C. Never work in a poorly ventilated area. Toxic fumes even in mild concentrations can knock you out. Be sure you have adequate ventilation before you start work in the laboratory.

D. Smoking and eating should be avoided when working with infectious materials such as wastewater and sludge. Never use laboratory glassware for serving the food.

E. Always use the proper type of equipment for handling hot containers, such as protective gloves, tongs, clothing, glasses, etc.

F. Where cylinders of oxygen or other compressed gases are used in the laboratory, they should be stored in separated and ventilated sections. They should be chained or clamped in an upright position while being used. The protective caps should never be removed until the cylinder is set and clamped in place, ready for attachment of valve gage and connections. Always use fittings approved for the cylinder being used and carefully follow instructions.

G. In working in the plant, be careful around:

   (1) Digesters--Do not smoke.

   (2) Chlorinators--Be aware of chlorine leaks. Chlorine may be detected by its odor, or a white mist will form near a rag soaked in ammonia.

   (3) Power and Blower--Wear ear plugs or ear covers if working over one hour in engine room.

   (4) Open Wastewater Tanks--Be careful; don't fall in.

   (5) Closed Wastewater Tanks--Avoid running over tank covers by foot or vehicle.

   (6) In Tanks or Near Construction--Wear hard hats.

14.21 Personal Hygiene for Wastewater Treatment Plant Personnel

Although it is highly unlikely that personnel can contract diseases by working in wastewater treatment plants, such a possibility does exist with certain diseases.

1. Some diseases are contracted through breaks in the skin, cuts, or puncture wounds. In such cases the bacteria causing the disease may be covered over and trapped by flesh, creating a suitable anaerobic environment[4] in which the bacteria may thrive and spread throughout the body.

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[4] Anaerobic Environment (AN-air-O-bick). A condition in which "free" or dissolved oxygen is not present.

For protection against diseases contracted through breaks in the skin, cuts, or puncture wounds, everyone working in or around wastewater must receive immunization from tetanus. Immunization must be received before the infection occurs. To prevent diseases from entering open wounds, care must be taken to keep wounds protected either with band aids or, if necessary, with rubber gloves or waterproof protective clothing.

2. Diseases that may be contracted through the gastrointestinal system or through the mouth are typhoid, cholera, dysentery, amebiases, worms, salmonella, infectious hepatitis, and polio virus. These diseases are transmitted by the infected wastewater materials being ingested or swallowed by careless persons. The best protection against these diseases is furnished by thorough cleansing. Hands, face, and body should be thoroughly washed with soap and water, particularly the hands, in order to prevent the transfer of any unsanitary materials or germs to the mouth while eating. A change of working clothes into street clothes before leaving work is highly recommended to prevent carrying unsanitary materials to the employee's home. Personal hygiene, thorough cleansing, and washing of the hands are effective means of protection.

   Immunization is provided for typhoid and polio. Little is known about infectious hepatitis except that it can be transmitted by wastewater. It is frequently associated with gross wastewater pollution.

3. Diseases that may be contracted by breathing contaminated air include (1) tuberculosis, (2) infectious hepatitis, and (3) San Joaquin fever. There has been no past evidence to indicate the transmission of tuberculosis through the air at wastewater treatment plants. However, there was one case of tuberculosis being contracted by an employee who fell into wastewater and, while swimming, inhaled wastewater into his lungs. San Joaquin fever is caused by a fungus which may be present in wastewater. However, there is no record of operators contracting the disease while on the job.

The best insurance against these diseases is proper personal hygiene and immunization. Your plant should have an immunization program against (1) tetanus, (2) typhoid, (3) polio, and (4) smallpox (although smallpox is not related to wastewater). The immunizations should be provided to protect you. Check with your local or state health department for recommendations regarding immunization.

In the washing of hands, the kind of soap is less important than the thorough use of the soap. (Special disinfectant soaps are not essential.)

The use of protective clothing is very important, particularly gloves and boots. The protection of wounds and cuts is also important. Report injuries and take care of them.

The responsibility rests upon you.

There is no absolute insurance against contraction of disease in a wastewater treatment plant. However, the likelihood of transmission is practically negligible. There appears to be no special risk in working at treatment plants. In fact, operators may receive a natural immunization by working in this environment.

QUESTIONS

14.2A Why should you always use a rubber bulb to pipette wastewater or polluted water?

14.2B Why are inoculations against disease recommended for people working around wastewater?

14.2C What would you do if you spilled a concentrated acid on your hand?

14.2D True or False: You may add acid to water, but never water to acid.

14.2E If you are working in a wastewater treatment plant, why should you change your clothes before going home at night?

14.3 SAMPLING, by Joe Nagano, from California Water Pollution Control Association Operators Laboratory Manual

14.30 Importance

Before any laboratory tests are performed, it is highly important to obtain a proper, representative sample. Without a representative sample, a test should not even be attempted because the test result will be incorrect and meaningless. A laboratory test without a good sample will most likely lead to erroneous conclusions and confusion. The largest errors produced in laboratory tests are usually caused by improper sampling, poor preservation, or lack of enough mixing during compositing[5] and testing.

14.31 Accuracy of Laboratory Equipment

Laboratory equipment, in itself, is generally quite accurate. Analytical balances weigh to 0.1 milligram. Graduated cylinders, pipettes, and burettes usually measure to 1% accuracy, so that the errors introduced by these items should total less than 5%, and under the worst possible conditions only 10%. Under ideal conditions let us assume that a test of raw wastewater for suspended solids should run about 300 mg/1. Because of the previously mentioned equipment or apparatus variables, the value may actually range from 270 to 330 mg/1. Results in this range are reasonable for operation. Other less obvious factors are usually present which make it quite possible to obtain results which are 25, 50, or even 100% in error, unless certain precautions are taken. Some examples will illustrate how these errors are produced.

The City of Los Angeles Terminal Island Treatment Plant is a primary treatment facility with a flow of 8 million gallons per day. It has an aerated grit chamber, two circular 85-foot clarifiers of 750,000 gallon capacity, and two digesters 100 and 75 feet in diameter.

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[5] Composite (Proportional) Samples (com-POZ-it). Samples collected at regular intervals in proportion to the existing flow and then combined to form a sample representative of the entire period of flow over a given period of time.

Monthly summary calculations based upon the suspended solids test showed that about 8,000 pounds of suspended solids were being captured per day during sedimentation assuming 200 mg/l for the influent and 100 mg/l for the effluent. However, it also appeared that 12,000 pounds per day of raw sludge solids were being pumped out of the clarifier and to the digester. Obviously, if sampling and analyses had been perfect, these weights would have balanced. The capture should equal the removal of solids. A study was made to determine why the variance in these values was so great. It would seem logical to expect that the problem could be due to (1) incorrect testing procedures, (2) poor sampling, (3) incorrect metering of the wastewater or sludge flow, or (4) any combination of the three or all of them.

In the first case, the equipment was in excellent condition. The operator was a conscientious and able employee who was found to have carried out the laboratory procedures carefully and who had previously run successful tests on comparative samples. It was concluded that the equipment and test procedures were completely satisfactory.

14.32 Selection of a Good Sampling Point to Obtain a Representative Sample

A survey was then made to determine if sampling stations were in need of relocation. By using Imhoff cones and running settleable solids tests along the influent channel and the aerated grit chamber, one could quickly recognize that the best mixed and most representative samples were to be taken from the aerated grit chamber rather than the influent channel.

The settleable solids ran 13 ml/l in the aerated grit chamber against 10 ml/l in the channel. By the simple process of determining the best sampling station, the suspended solids value in the influent was corrected from 200 mg/l to the more representative 300 mg/l. Calculations, using the correct figures, changed the solids capture from 8,000 pounds to 12,000 pounds per day and a balance was obtained.

This study clearly illustrates the importance of selecting a good sampling point in securing a truly representative sample. It emphasizes the point that even though a test is accurately performed, the result may be entirely erroneous and meaningless insofar as use for process control is concerned, unless a good representative sample is taken. Furthermore, a good sample is highly dependent upon the sampling station. Whenever possible, select a place where mixing is thorough and the wastewater quality is uniform. As the solids concentration increases, above about 200 mg/l, mixing becomes even more significant because the wastewater solids will tend to separate rapidly with the heavier solids settling toward the bottom, the lighter solids in the middle, and the floatables rising toward the surface. If, as is usual, a one-gallon portion is taken as representative of a million-gallon flow, the job of sample location and sampling must be taken seriously.

14.33 Time of Sampling

Let us consider next the time and frequency of sampling. In carrying out a testing program, particularly where personnel and time are limited due to the press of operational responsibilities, testing may necessarily be restricted to about one test day per week. If the operator should decide to start his tests early in the week, by taking samples early on Monday morning he may wind up with some very odd results.

One such incident will be cited. During a test for ABS (alkyl benzene sulfonate), samples were taken early on Monday morning and rushed into the laboratory for testing. Due to the detention time in the sewers, these wastewater samples actually represented Sunday flow on the graveyard shift, the weakest wastewater obtainable. The ABS content was only 1 mg/l, whereas it would normally run 8 to 10 mg/l. So the time and day of sampling is quite important, and the samples should be taken to represent typical weekdays or even varied from day to day within the week for a good cross-section of the characteristics of the wastewater.

14.34 Compositing and Preservation of Samples

Since the wastewater quality changes from moment to moment and hour to hour, the best results would be obtained by using some sort of continuous sampler-analyzer. However, since operators are usually the sampler-analyzer, continuous analysis would leave little time for anything but sampling and testing. Except for tests which cannot wait due to rapid chemical or biological change of the sample, such as tests for dissolved oxygen and sulfides, a fair compromise may be reached by taking samples throughout the day at hourly or two-hour intervals.

When the samples are taken, they should be immediately refrigerated to preserve them from continued bacterial decomposition. When all of the samples have been collected for a 24-hour period, the samples from a specific location should be combined or composited together according to flow to form a single 24-hour composite sample.

To prepare a composite sample, (1) the rate of wastewater flow must be metered and (2) each grab sample must then be taken and measured out in direct proportion to the volume of flow at that time. For example, Table I illustrates the hourly flow and sample volume to be measured out for a 12-hour proportional composite sample.

TABLE I

DATA COLLECTED TO PREPARE PROPORTIONAL COMPOSITE SAMPLE

Time	Flow MGD	Factor	Sample Vol	Time	Flow MGD	Factor	Sample Vol
6 AM	0.2	100	20	12 N	1.5	100	150
7 AM	0.4	100	40	1 PM	1.2	100	120
8 AM	0.6	100	60	2 PM	1.0	100	100
9 AM	1.0	100	100	3 PM	1.0	100	100
10 AM	1.2	100	120	4 PM	1.0	100	100
11 AM	1.4	100	140	5 PM	0.9	100	90
							1140

A sample composited in this manner would total 1140 ml.

Large wastewater solids should be excluded from a sample, particularly those greater than one-quarter inch in diameter.

A very important point should be emphasized. During compositing and at the exact moment of testing, the samples must be vigorously remixed so that they will be of the same composition and as well mixed as when they were originally sampled. Sometimes such remixing may become lax, so that all the solids are not uniformly suspended. Lack of mixing can cause low results in samples of solids that settle out rapidly, such as those in activated sludge or raw wastewater. Samples must therefore be mixed thoroughly and poured quickly before any settling occurs. If this is not done, errors of 25 to 50% may easily occur. For example, on the same mixed liquor sample, one person may find 3,000 mg/l suspended solids while another person may determine that there are only 2,000 mg/l due to poor mixing. When such a composite sample is tested, a reasonably accurate measurement of the quality of the day's flow can be made.

If a 24-hour sampling program is not possible, perhaps due to insufficient personnel or the absence of a night shift, single representative samples should be taken at a time when typical characteristic qualities are present in the wastewater. The samples should be taken in accordance with the detention time required for treatment. For example, this period may exist between 10 AM and 5 PM for the sampling of raw influent. If a sample is taken at 12 Noon, other samples should be taken in accordance with the detention periods of the serial processes of treatment in order to follow this slug of wastewater or plug flow. In primary settling, if the detention time in the primaries is two hours, the primary effluent should be sampled at 2 PM. If the detention time in the succeeding secondary treatment process required three hours, this sample should be taken at 5 PM.

14.35 Sludge Sampling

In sampling raw sludge and feeding a digester, a few important points should be kept in mind as shown in the following illustrative table.

For raw sludge from a primary clarifier at Los Angeles' Terminal Island Plant, the sludge solids varied considerably with pumping time as shown by samples withdrawn every one-half minute.

TABLE II

DECREASE IN PERCENT TOTAL SOLIDS DURING PUMPING

Pumping Time In Minutes	Total Solids Percent	Cumulative Solids Average
0.5	7.0	7.0
1.0	7.1	7.1
1.5	7.4	7.2
2.0	7.3	7.2
2.5	6.7	7.1
3.0	5.3	6.8
3.5	4.0	6.4
4.0	2.3	5.9
4.5	2.0	5.5
5.0	1.5	5.1

a. Table II shows that the solids were heavy during the first 2.5 minutes, and thereafter rapidly became thinner and watery. Since sludge solids should be fed to a digester with solids as heavy as possible and a minimum of water, the pumping should probably have been stopped at about 3 minutes. After 3 minutes, the water content did become greater that desirable.

b. In sampling this sludge, the sample should be taken as a composite by mixing small equal portions taken every 0.5 minutes during pumping. If only a single portion of sludge is taken for the sample, there is a chance that the sludge sample may be too thick or too thin, depending upon the moment the sample is taken. A composite sample will prevent this possibility.

c. It should also be emphasized again that as a sludge sample stands, the solids and liquid separate due to gasification and flotation or settling of the solids, and that it is absolutely necessary to thoroughly remix the sample back into its original form as a mixture before pouring it for a test.

d. When individual samples are taken at regular intervals in this manner, they should be carefully preserved to prevent sample deterioration by bacterial action. Refrigeration is an excellent method of preservation and is generally preferable to chemicals since chemicals may interfere with tests such as BOD and COD.

14.36 Sampling Devices

Automatic sampling devices are wonderful timesavers and should be employed where possible. However, like anything automatic, problems of which the operator should be aware do arise in their use. Sample lines to auto-samplers may build up growths which may periodically slough off and contaminate the sample with a high solids content. Very regular cleanout of the intake line is required. Another problem occurred at Los Angeles' Hyperion Plant when the reservoir for the automatic sampler was attacked by sulfides. Metal sulfides flaked off and entered the sample container producing misleading high solids results. The reservoir was cleaned and coated with coal-tar epoxy and little further difficulty has been experienced.

Manual sampling equipment includes dippers, weighted bottles, hand-operated pumps, and cross-section samplers. Dippers consist of wide-mouth corrosion resistant containers (such as cans or jars) on long handles that collect a sample for testing. A weighted bottle is a collection container which is lowered to a desired depth. At this location a cord or wire removes the bottle stopper so the bottle can be filled. Sampling pumps allow the inlet to the suction hose to be lowered to the sampling depth. Cross-sectional samplers are used to sample where the wastewater and sludge may be in layers, such as in a digester or clarifier. The sampler consists of a tube, open at both ends, that is lowered at the sampling location. When the tube is at the proper depth, the ends of the tube are closed and a sample is obtained from different layers.

Many operators build their own sampler (Fig. 14.1) using the material described below:

1. Sampling Bucket. A coffee can attached to an eight-foot length of 1/2-inch electrical conduit or a wooden broom handle with a 1/4-inch diameter spring in a four-inch loop.

2. Sampling Bottle. Plastic bottle with rubber stopper equipped with two 3/8-inch glass tubes, one ending near bottom of bottle to allow sample to enter and the other ending at the bottom of the stopper to allow the air in the bottle to escape while the sample is filling the bottle.

For sample containers, wide-mouth plastic bottles are recommended. Plastic bottles, though somewhat expensive initially, not only greatly reduce the problem of breakage and metal contamination, but are much safer to use. The wide-mouth bottles ease the washing problem. For regular samples, sets of plastic bottles bearing identification labels should be used.

14.37 Summary

1. Representative samples must be taken before any tests are made.

2. Select a good sampling location.

3. Collect samples and preserve them by refrigeration.

4. If possible, prepare 24-hour composite samples. Mix samples thoroughly before compositing and at the time of the test.

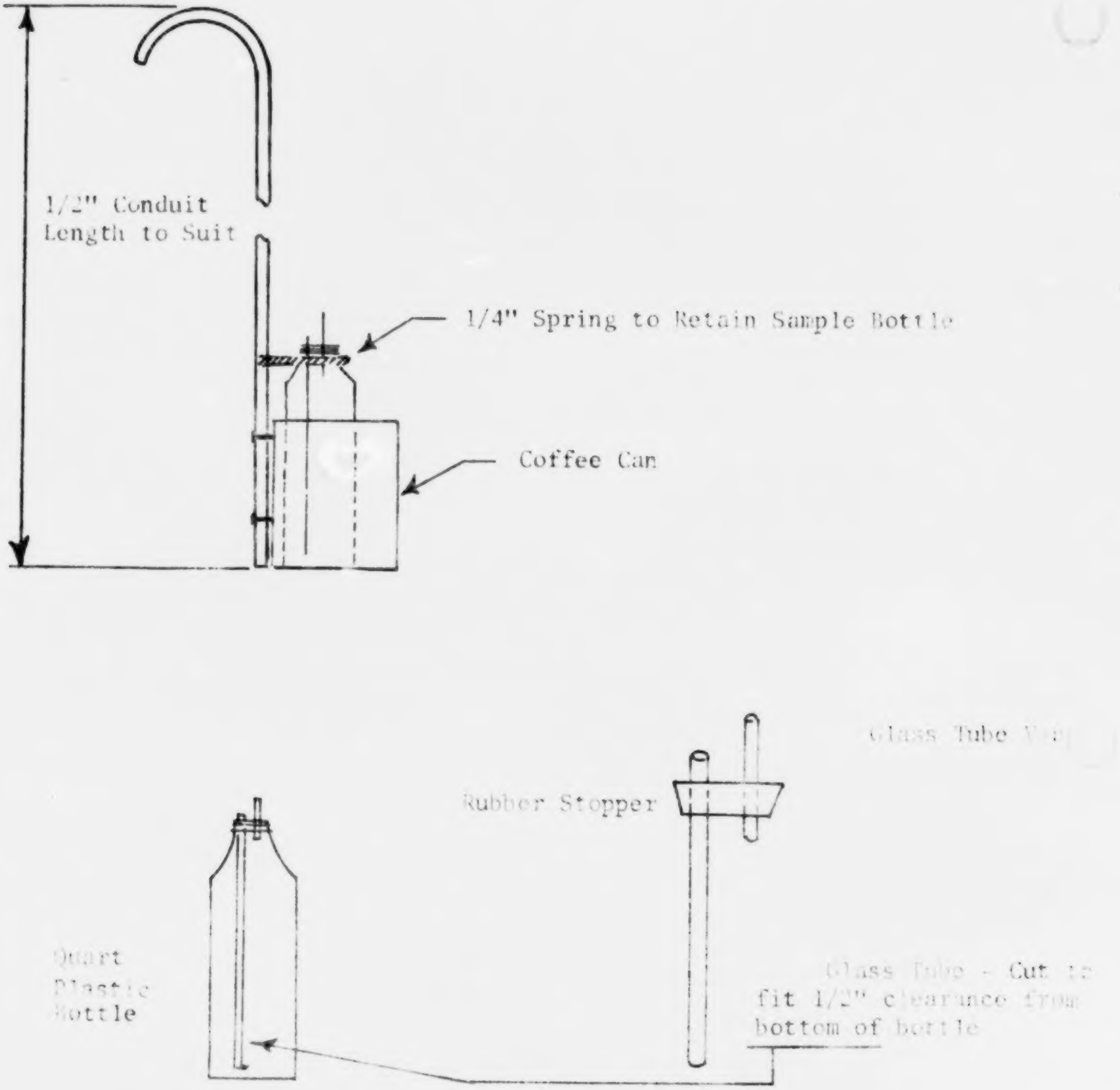

1/2" Conduit
Length to Suit
1/4" Spring to Retain Sample Bottle
Coffee Can
Glass Tube Vent
Rubber Stopper
Quart
Plastic
Bottle
Glass Tube - Cut to
fit 1/2" clearance from
bottom of bottle

Fig. 14.1 Sampling bottle

QUESTIONS

14.3A What are the largest sources of errors found in laboratory results?

14.3B Why must a representative sample be collected?

14.3C How would you prepare a proportional composite sample?

14.4 LABORATORY WORK SHEET

All laboratory results should be recorded immediately after a sample has been measured. There is no standard laboratory form; however, your plant or the agency that regulates your discharge may have a preferred form. Figure 14.2 is a typical laboratory work sheet (sometimes called a bench sheet) and will be referred to throughout the chapter.

PLANT ______________________

DATE ______________________

SUSPENDED SOLIDS & DISSOLVED SOLIDS

SAMPLE							
Crucible							
Ml Sample							
Wt Dry & Dish Wt Dish							
Wt Dry							
mg/l = $\frac{\text{Wt Dry, gm} \times 1{,}000{,}000}{\text{Ml Sample}}$							
Wt Dish & Dry Wt Dish & Ash							
Wt Volatile							
% Vol = $\frac{\text{Wt Vol}}{\text{Wt Dry}} \times 100\%$							

BOD

# Blank ______________

SAMPLE									
DO Sample									
Bottle #									
% Sample									
Blank or adj blank									
DO after incubation Depletion, 5 days									
Dep %									

Nitrate NO_3

Sample ______________ ______________

Graph Reading ______________ ______________

Sett. Solids

Sample ______________ ______________

Direct Ml/l ______________ ______________

COD

Sample ______________ ______________ ______________

Blank Titration ______________ ______________ ______________

Sample Titration ______________ ______________ ______________

Depletion ______________ ______________ ______________

mg/l = $\frac{\text{Dep} \times N \text{ FAS} \times 8000}{\text{Ml Sample}}$ ______________ ______________ ______________

Fig. 14.2 Typical laboratory work sheet

TOTAL SOLIDS

SAMPLE						
Dish No.						
Wt Dish & Wet						
Wt Dish						
Wt Wet						
Wt Dish + Dry						
Wt Dish						
Wt Dry						
% Solids = $\frac{\text{Wt Dry}}{\text{Wt Wet}} \times 100\%$						
Wt Dish + Dry						
Wt Dish + Ash						
Wt Volatile						
% Volatile = $\frac{\text{Wt Vol}}{\text{Wt Dry}} \times 100\%$						
pH						
Vol. Acid						
Alkalinity as $CaCO_3$						

Grease (Soxlet) ________________

Sample ________________

Ml Sample ________________

Wt Flask + Grease

Wt Flask ________________

Wt Grease ________________

mg/l = $\frac{\text{Wt Grease, mg} \times 1{,}000}{\text{Ml Sample}}$ ________________

H_2S (Gas) (Starch-Iodine)

Blank ________ Ml

Sample ________ Ml

Diff ________ Ml

Diff x .68 ________ mg/l

mg/l x 43.6 ________ grain/100 cu ft

Fig. 14.2 Typical laboratory work sheet (continued)

END OF LESSON 1 OF 8 LESSONS

on

Laboratory Procedures and Chemistry

EXPLANATION OF DISCUSSION AND REVIEW QUESTIONS

Work this portion of the discussion and review questions after you have completed answering the questions in Lesson 1. At the end of each lesson in this chapter you will find some discussion and review questions that you should complete before continuing.

The purpose of these questions is to indicate to you how well you understand the material in this chapter.

DISCUSSION AND REVIEW QUESTIONS

(Lesson 1 of 8 Lessons)

Chapter 14. Laboratory Procedures and Chemistry

Name ______________________________ Date ____________

Write the answers to these questions in your notebook before continuing.

1. What precautions should an operator take to protect himself from diseases when working in a wastewater treatment plant?
2. Why should work with certain chemicals be conducted under a ventilated laboratory hood?
3. What is meant by a representative sample?
4. How would you obtain a representative sample?

CHAPTER 14. LABORATORY PROCEDURES AND CHEMISTRY

(Lesson 2 of 8 Lessons)

14.5 PLANT CONTROL TESTS

Tests in this section are listed in alphabetical order. Many of the tests are conducted at primary, secondary, and advanced wastewater treatment plants. Certain tests are commonly used to control digester operation and activated sludge plants. Typical plant and special plant control tests are summarized below.

A. Typical Plant Control Tests

TEST NO.	TITLE
2	Biochemical Oxygen Demand or BOD, Procedure with DO
4	Chemical Oxygen Demand or COD
5	Chlorine Residual
6	Clarity
7	Coliform Group Bacteria
8	Dissolved Oxygen or DO
9	Hydrogen Sulfide
10	pH
12	Settleable Solids
16	Suspended Solids (Gooch Crucible)
17	Temperature (Wastewater)

B. Digester Control Tests

TEST NO.	TITLE
1	Alkalinity, Procedure with Volatile Acids
3	Carbon Dioxide (CO_2) in Digester Gas
14	Sludge Dewatering Characteristics
15	Supernatant Graduate Evaluation
17	Temperature (Digester Sludge)
20	Volatile Acids
21	Total and Volatile Solids (Sludge)

C. Activated Sludge Control Tests

TEST NO.	TITLE
8	Dissolved Oxygen (In Aerator)
11	Settleability
13	Sludge Age
11	Sludge Density Index (SDI)
11	Sludge Volume Index (SVI)
16	Suspended Solids (Centrifuge)

1. Total Alkalinity

The alkalinity test is located with the volatile acid test because the volatile acid/alkalinity relationship is critical in the successful operation of sludge digesters.

2. Biochemical Oxygen Demand or BOD

The BOD test is placed with the dissolved oxygen (DO) test because to measure the rate of oxygen uptake in the BOD test, the DO must be measured.

3. Carbon Dioxide (CO_2) in Digester Gas

A. Discussion

Changes in the anaerobic sludge digestion process will be observed in the gas quality and are usually noted after the volatile acids or volatile acid/alkalinity relationship starts to increase. The CO_2 content of a properly operating digester will range from 30% to 40% by volume. If the percent is above 44%, the gas will not burn. The easiest test procedure for determining this change is with a CO_2 analyzer.

B. What is Tested?

Sample	Preferred
CO_2 in Digester Gas	30% - 35% by Volume

METHOD A

C. Apparatus

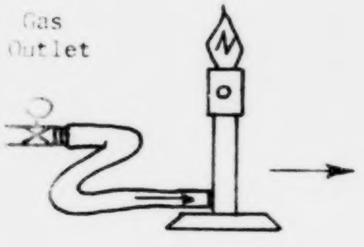
1. One Bunsen burner
2. Plastic tubing
3. 100 ml graduated cylinder
4. 250 ml beaker

D. Reagents

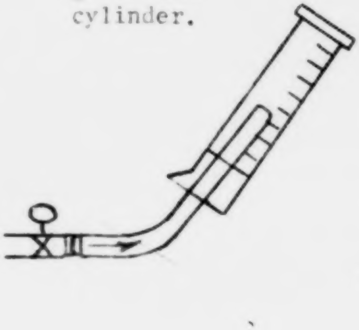
CO_2 Absorbent (KOH). Add 500 g potassium hydroxide (KOH) per liter of water.

E. Outline of Procedure

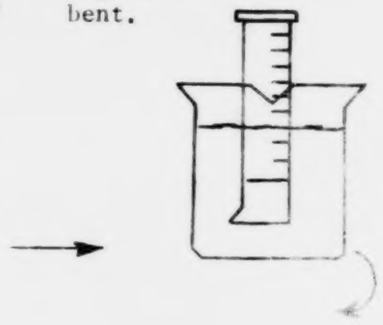
1. Clean out sampling line by allowing gas from sampling outlet to burn until line is full of gas from digester.



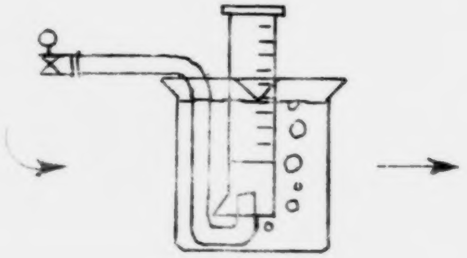
2. Displace air in graduated cylinder.



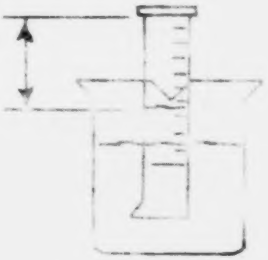
3. Place graduate upside down in beaker containing CO_2 absorbent.



4. Insert hose in graduate and run gas for 60 seconds.



5. Remove hose from graduate and then turn off gas. Wait 10 minutes.



6. Read volume of gas removed to nearest ml.

PRECAUTIONS

1. Avoid any open flames near the digester.
2. Work in a well ventilated area to avoid the formation of explosive mixtures of methane gas.
3. If your gas sampling outlet is on top of your digester, turn on outlet and vent the gas to the atmosphere for several minutes to clear the line of old gas. Start with step 2, displace air in graduated cylinder. NEVER ALLOW ANY SMOKING OR FLAMES NEAR THE DIGESTER AT ANY TIME.

PROCEDURE

1. Measure total volume of a 100 ml graduate by filling it to the top with water (approximately 125 ml). Record this volume.
2. Pour approximately 125 ml of CO_2 absorbent in a 250 ml beaker.

   CAUTION: Do not get any of this chemical on your skin or clothes. Wash immediately with running water until slippery feeling is gone or severe burns can occur.

3. Collect a representative sample of gas from the gas dome on the digester, a hot water heater using digester gas to heat the sludge, or any other gas outlet. Before collecting the sample for the test, attach one end of a gas hose to the gas outlet and the other end to a Bunsen burner. Turn on the gas, ignite the burner, and allow it to burn digester gas for a sufficient length of time to insure collecting a representative gas sample.
4. With gas running through hose from gas sampling outlet, place hose inside inverted calibrated graduated cylinder and allow digester gas to displace air in graduate. Turn off gas.

   CAUTION: The proper mixture of digester gas and air is explosive when exposed to a flame.

5. Place graduate full of digester gas upside down in beaker containing CO_2 absorbent.
6. Insert gas hose inside upside down graduate.
7. Turn on gas, but do not blow out liquid. Run gas for at least 60 seconds.
8. Carefully remove hose from graduate with gas still running.
9. Immediately turn off gas.
10. Wait for ten minutes and shake gently. If liquid continues to rise, wait until it stops.
11. Read gas remaining in graduate to nearest ml. (Fig. 14.3)

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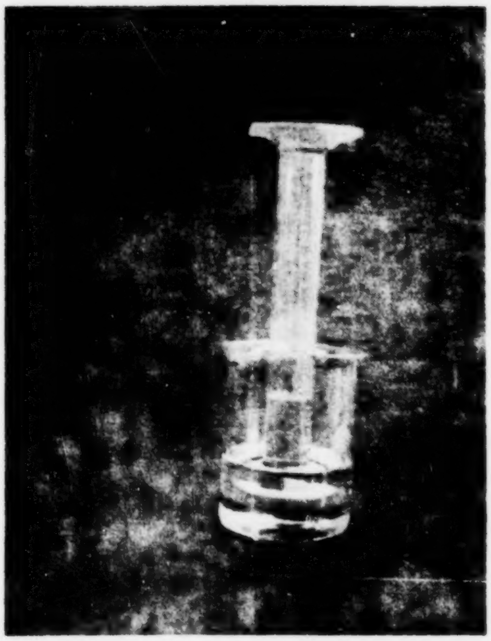


Fig. 14.3 CO_2 measurement using inverted graduated cylinder

F. Example

Total Volume of Graduate = 126 ml

Gas Remaining in Graduate = 80 ml

G. Calculation

$$\% \ CO_2 = \frac{(\text{Total Volume, ml} - \text{Gas Remaining, ml})}{\text{Total Volume, ml}} \times 100\%$$

$$= \frac{(126 \text{ ml} - 80 \text{ ml})}{126 \text{ ml}} \times 100\%$$

$$= \frac{46}{126} \times 100\%$$

$$= 37\%$$

$$\begin{array}{r} .365 \\ 126\,\overline{)\,46.0} \\ \underline{37\ 8} \\ 8\ 20 \\ \underline{7\ 56} \\ 640 \\ 630 \end{array}$$

METHOD B

(ORSAT)

The Orsat gas analyzer can measure the concentrations of carbon dioxide, oxygen, and methane by volume in digester gas. To analyze digester gas by the Orsat method, follow equipment manufacturer's instructions. This procedure is not recommended for the inexperienced operator.

QUESTIONS

3.A What are the dangers involved in running the CO_2 in digester gas test?

3.B What is the percent CO_2 in a digester gas if the total volume of the graduated cylinder is 128 ml and the gas remaining in the cylinder after the test is 73 ml?

4. Chemical Oxygen Demand or COD

A. Discussion

COD is a good estimate of the first-stage oxygen demand for most municipal wastewaters. An advantage of the COD test over the BOD test is that you do not have to wait for five days for the results. The COD test also is used to measure the strength of wastes that are too toxic for the BOD test. COD is usually higher than the BOD, but the amount will vary from waste to waste. The method related here is a quick, effective measure of the strength of a waste.

B. What is Tested?

Sample	Common Range, mg/l
Influent	200 - 400
Effluent	40 - 80
Industrial Waste	200 - 4000

C. Apparatus

Two 50 ml graduated cylinders

10 ml pipette

50 ml burette

Boiling flask

Reflux condenser

Hot plate

D. Reagents

1. Standard potassium dichromate ($K_2Cr_2O_7$) 0.250 N. Dissolve 12.259 g dried $K_2Cr_2O_7$ in distilled water and make up to 1 liter.

2. Surfuric acid-silver sulfate reagent. Add 22 g of silver sulfate (Ag_2SO_4) to a 9-lb bottle of concentrated sulfuric acid (H_2SO_4). It takes one to two days to dissolve.

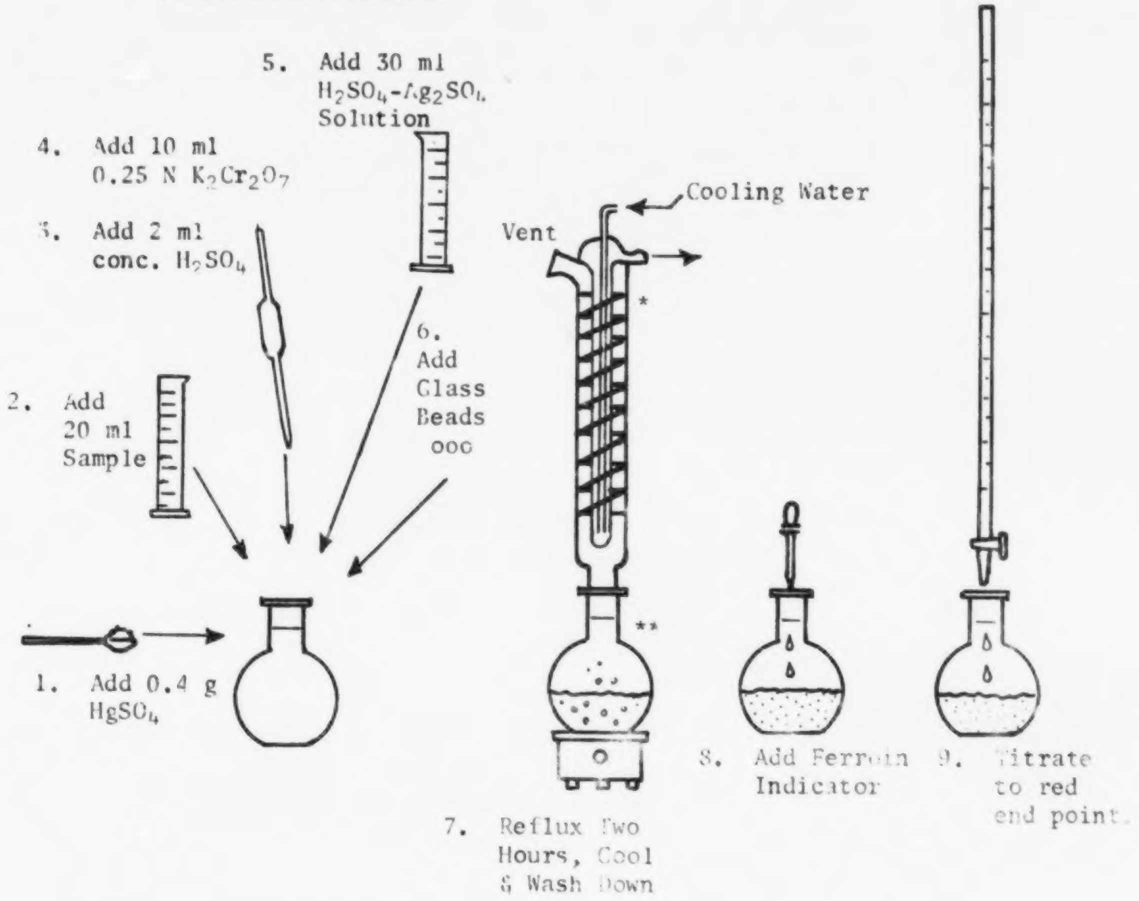
3. Standard ferrous ammonium sulfate solution, 0.25 N. Dissolve 98 g $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in distilled water, add 20 ml concentrated H_2SO_4, cool and dilute to 1 liter. This solution is unstable and must be standardized daily.

4. Ferroin Indicator. Dissolve 1.485 g of 1,10 phenanthroline ($C_{12}H_8N_2 \cdot H_2O$), together with 0.695 g ferrous sulfate crystals ($FeSO_4 \cdot 7H_2O$), in water and make up to 100 ml.

5. Silver sulfate, reagent powder.

6. Mercuric sulfate ($HgSO_4$) analytical grade crystals.

E. Outline of Procedure


5. Add 30 ml H_2SO_4-Ag_2SO_4 Solution
4. Add 10 ml 0.25 N $K_2Cr_2O_7$
3. Add 2 ml conc. H_2SO_4
2. Add 20 ml Sample
6. Add Glass Beads ooo
1. Add 0.4 g $HgSO_4$
Cooling Water
Vent
*
**
7. Reflux Two Hours, Cool & Wash Down
8. Add Ferroin Indicator
9. Titrate to red end point.

* Reflux condenser, Friedrichs, VWR - 23157-001
** Flask, boiling, flat bottom, VWR - 29113-068

PROCEDURE

1. Place 0.4 g mercuric sulfate into a 250 ml Erlenmeyer flask with a ground glass neck.

2. Measure 20.0 ml sample into the flask.

3. Add 2.0 ml concentrated sulfuric acid. Swirl until contents are well mixed.

4. Pipette 10.0 ml standard potassium dichromate solution into the flask.

5. Carefully add 30 ml sulfuric acid-silver sulfate reagent into the flask while swirling the flask. Use caution. Make sure contents of the flask are thoroughly mixed before heat is applied.

6. Add a few glass beads to reduce bumping and connect to condenser. The reflux mixture must be thoroughly mixed before heat is applied. If this is not done, local hot spots on bottom of flask may cause mixture to be blown out of flask.

7. Prepare a blank[5] by repeating above steps and by substituting distilled water for the sample.

8. Reflux samples and blank for two hours. (If sample mixture turns completely green, the sample was too strong. Dilute sample with distilled water and repeat above steps substituting diluted sample.)

9. While the samples and blank are refluxing, standardize the ferrous ammonium sulfate solution:

   a. Pipette 10.0 ml standard potassium dichromate solution into a 250 ml Erlenmeyer flask. Add about 100 ml of water.

   b. Add 30 ml concentrated H_2SO_4 with mixing. Let cool.

   c. Add 2-3 drops ferroin indicator, titrate with ferrous ammonium sulfate (FAS) solution. Color change of solution is from orange to greenish to red.

   ml FAS ________________________________

   Concentration Ratio, $R = \frac{10 \text{ ml } K_2Cr_2O_7}{\text{ml FAS}}$ = ____________

10. After refluxing mixture for two hours, wash down condenser. Let cool. Add distilled water to about 140 ml.

11. Titrate reflux mixtures with standard FAS.

    Blank - ml FAS ____________

    Sample - ml FAS ____________

---

[5] Blank. A bottle containing dilution water or distilled water, but the sample being tested is not added. Tests are frequently run on a sample and a blank and the differences compared.

F. Precautions

1. Wastewater sample should be well mixed. If large particles are present, sample should be homogenized.

2. Flasks and condensers should be clean and free from grease or other oxidizable materials, otherwise erratic results would be obtained.

3. The standard ferrous ammonium sulfate solution is unstable and should be standardized daily or each time the COD test is performed.

4. Use extreme caution in handling concentrated H_2SO_4. Spillage on skin or clothing should be immediately washed off and neutralized.

5. The solution must be well mixed before it is heated. If the acid is not completely mixed in the solution when it is heated, the mixture could spatter and some of it will pass out the vent, thus ruining the test.

6. Mercury sulfate is very toxic. Avoid skin contact and breathing of this chemical.

G. Example

1. Standardization of ferrous ammonium sulfate, FAS.

ml 0.25 N $K_2Cr_2O_7$ = 10.0

ml FAS = 11.0

$$\text{Concentration Ratio, R} = \frac{\text{ml } K_2Cr_2O_7}{\text{ml FAS}}$$

$$= \frac{10.0}{11.0}$$

2. Sample test.

Sample Taken = 20.0 ml

A = ml FAS used for blank = 10.0 ml

B = ml FAS used for sample = 3.0 ml

H. Calculation for COD

Method 1

$$\text{COD, mg/l} = (A - B) \times R \times 100$$

$$= (10.0 - 3.0)\ (10/11)\ (100)$$

$$= 635 \text{ mg/l}$$

Method 2 (According to Standard Methods)

$$\text{COD, mg/l} = \frac{(A - B) \times C \times 8000}{\text{ml Sample}}$$

where

C = Normality of FAS

N = Normality of $K_2Cr_2O_7$ Standard

$$C = \frac{\text{ml } K_2Cr_2O_7}{\text{ml FAS}} \times N$$

$$= \frac{10.0}{11.0} \times 0.25$$

$$= 0.227$$

$$\text{COD, mg/l} = \frac{(10.0 - 3.0)(0.227)(8000)}{20}$$

$$= 635 \text{ mg/l}$$

QUESTIONS

4.A What does the COD test measure?

4.B What are some of the advantages of the COD test over the BOD test?

5. Chlorine Residual

A. Discussion

A chlorine residual should be maintained in a plant effluent for disinfection purposes. The amount of residual remaining in the treated wastewater after passing through a contact basin or chamber may be related to the numbers of bacteria allowed in the effluent by regulatory agencies.

Method A (Iodometric) is used for samples containing wastewater, such as plant effluents or receiving waters. Method B (Deleted). Method C (Amperometric[7] Titration) gives the best results, but the titrator is expensive.

B. What is Tested?

Sample	Common Range, mg/l (After 30 Minutes)
Effluent	0.5 - 2.0 mg/l

C. Apparatus

METHOD A (Iodometric)

1. One 250 ml graduated cylinder
2. One 10 ml measuring pipette
3. One 500 ml Erlenmeyer flask
4. Two 5 ml measuring pipettes
5. One 50 ml Buret

---

[7] Amperometric (am-PURR-o-MET-rick). A method of measurement that records electric current flowing or generated, rather than recording voltage. Amperometric titration is an electrometric means of measuring concentrations of substances in water.

METHOD B (Orthotolidine-Arsenite or OTA)

One permanent glass color comparator
Three comparator cells

METHOD C (Amperometric Titration)

See Standard Methods

D. Reagents

METHOD A

1. Standard phenylarsine oxide solution, 0.00564 N. Dissolve approximately 0.8 g phenylarsine oxide powder in 150 ml 0.3 N NaOH solution. After settling, remove upper 110 ml of this solution into 800 ml distilled water and mix thoroughly. Adjust pH up to between 6 and 7 with 6 N HCl and dilute to 950 ml with distilled water. To standardize this solution accurately measure 5 to 10 ml of freshly standardized 0.0282 N iodine solution into a flask and add 1 ml KI solution. Titrate with phenylarsine oxide solution, using starch solution as an indicator. Adjust to exactly 0.00564 N and recheck against the standard iodine solution; 1.00 ml = 200 ug available chlorine. CAUTION: Toxic - avoid ingestion.

2. Potassium iodide, crystals.

3. Acetate buffer solution, pH 4.0. Dissolve 146 g anhydrous $NaC_2H_3O_2$, or 243 g $NaC_2H_3O_2 \cdot 3H_2O$, in 400 ml distilled water, add 480 g concentrated acetic acid, and dilute to 1 liter with distilled water.

4. Standard iodine titrant, 0.0282 N. Dissolve 25 g KI in a little distilled water in a 1-liter volumetric flask, add the proper amount of 0.1 N iodine solution exactly standardized to yield a 0.0282 N solution, and dilute to 1 liter. Store in amber bottles or in the dark, protecting the solution from direct sunlight at all times and keeping it from all contact with rubber.

5. Starch indicator. Make a thin paste of 6 g of potato starch in a small quantity of distilled water. Pour this paste into one liter of boiling, distilled water. Allow to boil for a few minutes, then settle overnight. Remove the clear supernatant and save; discard the rest. For preservation, add two drops of toluene ($C_6H_5CH_3$).

METHOD B

Deleted

METHOD C

See Standard Methods

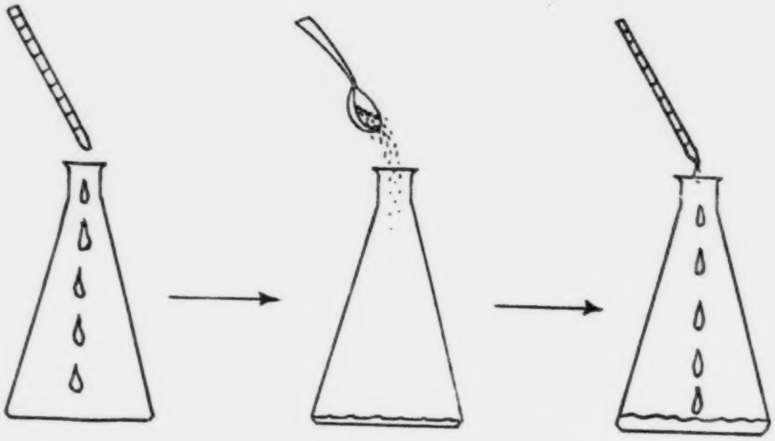
E. Procedure

METHOD A

1. Place 5.00 ml phenylarsine oxide solution to Erlenmeyer flask

2. Add excess KI (approx 1 g)

3. Add 4 ml acetate buffer solution

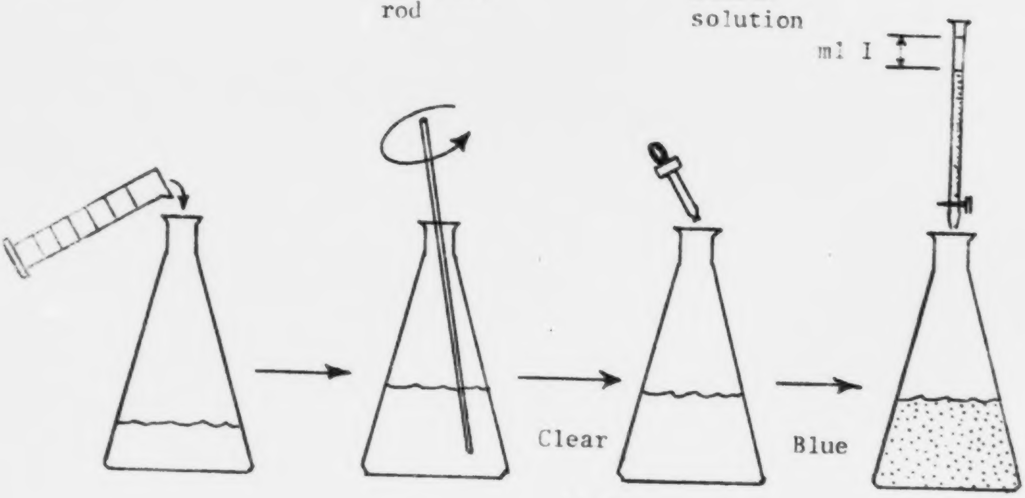


4. Add 200 ml sample

5. Mix with stirring rod

6. Add 1 ml starch solution

7. Titrate until blue color first appears and remains after mixing



METHOD A

1. Place 5.00 ml 0.00564 N phenylarsine oxide solution in an Erlenmeyer flask.
2. Add excess KI (approx. 1 g).
3. Add 4 ml acetate buffer solution, or enough to lower the pH to between 3.5 and 4.2.
4. Pour in 200 ml of sample.
5. Mix with a stirring rod.
6. Add 1 ml starch solution just before titration.
7. Titrate to the first appearance of blue color, which remains after complete mixing.

METHOD B

1. Label the three comparator cells "A," "B," and "C." Use 0.5 ml of orthotolidine reagent in 10-ml cells, 0.75 ml in 15-ml cells, and the same ratio for other volumes of sample. Use the same volume of arsenite solution as orthotolidine.
2. Add orthotolidine reagent to Cell A.
3. Add sample to mark on wall of Cell A. Mix quickly, and immediately (within 5 seconds) add arsenite solution. Mix quickly again and compare with color standards as rapidly as possible.

   Free available chlorine and
   interfering colors, A = ____________ mg/l

4. Add arsenite solution to Cell B.

5. Add sample to mark on wall of Cell B. Mix quickly, and immediately add orthotolidine reagent. Mix quickly again and compare with color standards as rapidly as possible.

   Interfering colors present in immediate reading, B_1 = ________ mg/l

6. Compare with color standards again in exactly 5 minutes.

   Interfering colors present in 5-minute reading, B_2 = ________ mg/l

7. Add orthotolidine reagent to Cell C.

8. Add sample to mark on wall of Cell C. Mix quickly and compare with color standards in exactly 5 minutes.

   Total amount of residual chlorine and interfering colors present, C = __________ mg/l

F. Examples and Calculations

Method A

Titration of a 200 ml sample required 0.4 ml of 0.0282 N I.

$$\text{Chlorine Residual, mg/l} = \frac{(1 - \text{ml I})\ 1000}{\text{Sample Volume, ml}}$$

$$= \frac{(1 - 0.4)\ (1000)}{200}$$

$$= (0.6)\ (5)$$

$$= 3.0\ \text{mg/l}$$

NOTE: The larger the ml of I used in the titration, the smaller the (1 - ml I) term and thus the lower the chlorine residual. This is why this test is sometimes called the back titration test for chlorine residual. If 1 ml of I is used in the titration, you have titrated back to a zero chlorine residual.

Method B

Results from the OTA test on a plant effluent.

$A = 0.5$ mg/l

$B_1 = 0.2$ mg/l

$B_2 = 0.3$ mg/l

$C = 1.4$ mg/l

Total Available Residual Chlorine, mg/l $= C - B_2$

$= 1.4$ mg/l $- 0.3$ mg/l

$= 1.1$ mg/l

Free Available Residual Chlorine, mg/l $= A - B_1$

$= 0.5$ mg/l $- 0.2$ mg/l

$= 0.3$ mg/l

Combined Available Residual Chlorine, mg/l = Total Available Residual Cl, mg/l - Free Available Residual Cl, mg/l

$= 1.1$ mg/l $- 0.3$ mg/l

$= 0.8$ mg/l

Total available residual chlorine consists of free available chlorine (HOCl and OCl^-) and combined available chlorine (chloramines--compounds formed by the reaction of chlorine with ammonia).

QUESTIONS

5.A Why should plant effluents be chlorinated?

5.B Discuss the important differences between the Iodometric titration, orthotolodine, and amperometric titration methods of measuring chlorine residual.

END OF LESSON 2 OF 8 LESSONS

on

Laboratory Procedures and Chemistry

Work the next portion of the discussion and review questions before continuing with Lesson 3.

DISCUSSION AND REVIEW QUESTIONS

(Lesson 2 of 8 Lessons)

Chapter 14. Laboratory Procedures and Chemistry

Name ____ Date ____________

Write the answers to these questions in your notebook. The problem numbering continues from Lesson 1.

5. How can you obtain a representative sample of digester gas?
6. Why is the COD test run?
7. Why should a chlorine residual be maintained in a plant effluent?

CHAPTER 14. LABORATORY PROCEDURES AND CHEMISTRY

(Lesson 3 of 8 Lessons)

6. Clarity

A. Discussion

All high quality effluents should have a clarity reading taken at high noon or some other specific time. This test is based on how far you can see through your plant effluent under similar conditions at the same time every day. The objective of the test is to indicate the clearness or clarity of the plant effluent. The test can be performed either in the lab by looking down through the effluent in a graduated cylinder, or in the field by looking down through the effluent in a clarifier or chlorine contact basin. Sometimes this test is referred to as a turbidity measurement, but you are interested in the clarity of your effluent.

B. What is Tested?

Sample	Common Range (Field Test)	
	Poor	Good
Secondary Clarifiers:		
Trickling Filter	1 ft	3 ft
Activated Sludge	3 ft	6 ft
Activated Sludge Blanket in Secondary Clarifier	1 ft	4 ft
Chlorine Contact Basins	1 ft	5 ft

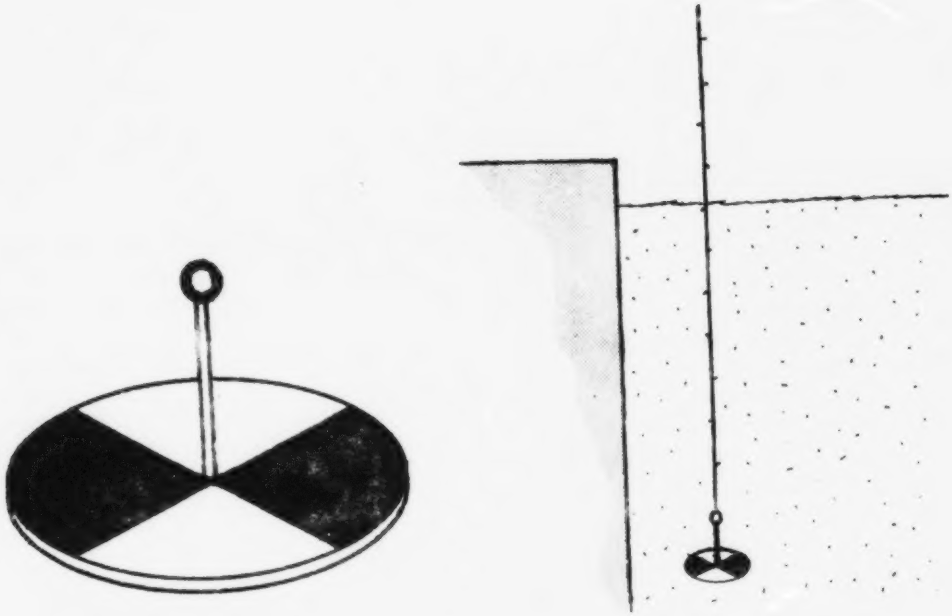
C. Apparatus

1. One clarity unit (Secchi (SECK-key) Disc) and attached cord marked in one-foot units.
2. One 1000 ml graduated cylinder
3. Hach Turbidimeter, Model 2100 A

D. Reagents

None

E. Procedures



1. Field Test. Tie end of marked nylon rope to handrail where tests will be run, for example, in final sedimentation unit. Always take tests at the same time each day for comparable results. Lower disc slowly until you just lose sight of it. Stop. Bring up slowly until just visible. Stop. Look at the marks on the rope to see the depth of water that you can see the disc through. Bring up disc and store. Record results.

2. Lab Test. Use a clean 1000 ml graduate. Fill with a well-mixed sample up to the 1000 ml mark. During every test the same lighting conditions in the lab should be maintained. Look down through the liquid in the cylinder and read the last visible number etched on the side of the graduate and record results.

3. Hach Turbidimeter. Follow manufacturer's instructions.

Whether you use one or each of these tests, you should run either test at the same time every day and under similar conditions for comparable results.

F. Example and Calculation

1. Each foot of depth is better clarity with Secchi disc.

2. Each 100 ml seen in depth is better clarity.

3. Turbidimeter reading indicates degree of clarity.

QUESTION

6.A What does the clarity test tell you about the quality of effluent?

6.B What happens when you attempt to measure clarity under different conditions, such as lighting and clarifier loadings?

7. Coliform Group Bacteria

A. Discussion

Coliform bacteria are measured to indicate the presence of bacteria originating in the intestines of warm-blooded animals. High coliform counts indicate the usefulness of water may have been impaired by fecal contamination. Coliform bacteria are considered harmless, but their presence may be indicative of the presence of disease-producing organisms that may be found with them.

B. What is Tested?

Sample	Usual Range, MPN/100 ml
Effluent:	
Primary	5,000 to 1,000,000
Nonchlorinated Secondary	>240,000
Chlorinated Secondary	50 to 500
Receiving Waters	1,000 to 1,000,000

C. Sampling Bottles

Polypropylene wide-mouthed bottles with 200 to 400 ml capacity are used to collect samples. Before sterilization by autoclave, add sodium thiosulfate (0.1 ml of a 10% solution per 4 ounce bottle) to the bottles to neutralize any chlorine residual in the samples. When filling bottles in the field, do not flush out sodium thiosulfate or contaminate sample or bottle. Fill bottles approximately three-quarters full, maintain at 4° C with ice during transport and start test in lab within eight hours after sampling.

D. Media Preparation

1. General Discussion

Careful media preparation is necessary to meaningful bacteriological testing. Attention must be given to the quality, mixing, and sterilization of the ingredients. The purpose of this care is to assure that if the bacteria being tested for are indeed present in a sample, every opportunity is presented for their development and ultimate identification. Much bacteriological identification is done by noting changes in the medium; consequently, the composition of the medium must be standardized. Much of the tedium of media preparation can be avoided by purchase of dehydrated media (Difco, BBL, or equivalent). The operator is advised to make use of these products; and, if only a limited amount of testing is to be done, consider using tubed, prepared media.

2. Glassware

All glassware must be thoroughly cleansed using a suitable detergent and hot water (160°F), rinsed with hot water (180°F) to remove all traces of residual detergent, and finally rinsed with distilled or deionized water.

3. Water

Only distilled water or demineralized water which has been tested and found free from traces of dissolved metals and bactericidal and inhibitory compounds may be used for preparation of culture media.

4. Buffered[8] Dilution Water

Prepare a stock solution by dissolving 34 grams of KH_2PO_4 in 500 ml distilled water, adjusting the pH to 7.2 with 1N NaOH. Prepare dilution water by adding 1.25 ml of the stock solution per liter of distilled water. This solution can be dispersed into various size dilution blanks or used as a sterile rinse water for the membrane filter test.

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[8] Buffer. A measure of the ability or capacity of a solution or liquid to neutralize acids or bases. This is a measure of the capacity of water or wastewater for offering a resistance to changes in the pH.

5. Coliform Test--Fermentation Tube Method

a. Lactose Broth or Lauryl Tryptose Broth

For the presumptive coliform test, dissolve the recommended amount of the dehydrated medium in distilled water. Dispense solution into fermentation tubes containing an inverted glass vial. Autoclave the capped tubes at 121°C for 15 minutes.

b. Brilliant Green Bile Lactose Broth

For the confirmed coliform test, dissolve 40 grams of the dehydrated medium in one liter of distilled water. Dispense and sterilize as with Lactose Broth.

c. Compensation for Diluting Effect of Samples

Large volumes of samples can dilute the medium in the fermentation tube. Use the concentrations listed below to compensate for diluting effects when using lauryl tryptose broth.

No. ml medium in tube	Ml of sample or dilution	Nominal concentration before inoculation	No. grams dehydrated medium per liter
10	0.1 to 1.0	1x	35.6
10	10	2x	71.2
20	10	1.5x	53.4
35	100	4x	137.3

6. Coliform Test-Elevated Temperature for Fecal Coliforms

EC Broth

For the fecal coliform test, dissolve 37 grams of the dehydrated medium in one liter of distilled water. Dispense and sterilize as with Lactose Broth.

7. Coliform Test--Membrane Filter Method

M-Endo Broth

Prepare this medium by dissolving 48 grams of the dehydrated product in one liter of distilled water which contains 20 ml of ethyl alcohol per liter. Heat solution to boiling only--DO NOT AUTOCLAVE. Prepared media should be stored in a refrigerator and used within 96 hours.

8. Autoclaving

Steam autoclaves are used for the sterilization of the liquid media and associated apparatus. They sterilize (killing of all organisms) at a relatively low temperature of 121°C within 15 minutes by utilizing moist heat.

Components of the media, particularly sugars such as lactose, may decompose at higher temperatures or longer heating times. For this reason adherence to time and temperature schedules is vital.

Autoclaves operate in a manner similar to the familiar kitchen pressure cooker:

1. Water is heated in a boiler to produce steam.
2. The steam is vented to drive out air.
3. The steam vent is closed when the air is gone.
4. Continued heat raises the pressure to 15 lbs/in^2 (at this pressure, pure steam has a temperature of 121°C).
5. The pressure is maintained for the required time.
6. The steam vent is opened and the steam is slowly vented until atmospheric pressure is reached. (Fast venting will cause the liquids to boil.)
7. Sterile material is removed to cool.

In autoclaving fermentation tubes, a vacuum is formed in the inner tubes. As the tubes cool, the inner tubes are filled with sterile medium. Capture of gas in this inner tube from the culture of bacteria is the evidence of fermentation.

E. Test for Coliform Bacteria

1. General Discussion

The test for coliform bacteria is used to measure the suitability of a water for human use. The test is not only useful in determining the bacterial quality of a finished water, but it can be used by the operator in the treatment plant to guide him in achieving a desired degree of treatment.

2. Multitube Fermentation Technique

Coliform bacteria are detected in water by placing portions of a sample of the water in lactose broth. Lactose broth is a standard bacteriological medium containing lactose (milk) sugar in tryptose broth. The coliform bacteria are those which will grow in this medium at 35°C temperature and ferment and produce gas from the sugar within 48 hours. Thus to detect these bacteria the operator need only inspect fermentation tubes for gas. In practice, multiple fermentation tubes are used in a decimal dilution for each sample.

3. Materials Needed

1. Fifteen sterile tubes of lactose broth are needed for each sample.
2. Use five tubes for each dilution.
3. Dilution tubes or blanks containing 9 ml or 99 ml of sterile buffered distilled water.
4. Quantity of one and 10 ml sterile pipettes.

4. Technique for Inoculation and/or Dilution of Sample (Fig. 14.4)

All inoculations and dilutions of wastewater specimens must be accurate and should be made so that no contaminants from the air, equipment, clothes or fingers reach the specimen, either directly or by way of the contaminated pipette.

1. Shake the specimen bottle vigorously 20 times before removing sample volumes.

2. Into the first five lactose tubes pipette 1.0 ml of sample directly into each tube. (It is important to realize that the sample volume applied to the first 5 tubes will depend upon the type of water being tested. The sample volume applied to each tube can vary from 10 ml (or more) for high quality waters to as low as 10^5 or 0.00001 ml (applied as 1ml of a diluted sample) for raw wastewater specimens).

Note: When delivering the sample into the culture medium, deliver sample portions of 1ml or less down into the culture tube near the surface of the medium. Do not deliver small sample volumes at the top of the tube and allow them to run down inside the tube; too much of the sample will fail to reach the culture medium.

Note: Use 10 ml pipette for 10 ml sample portions, and 1 ml pipette for portions of 1 ml or less. Handle sterile pipettes only near the mouthpiece, and protect the delivery end from external contamination.

3. Pipette 1/10 ml or 0.1 ml of raw sample into each of the next 5 lactose broth tubes. This makes a 0.1 dilution.

4. To make the 0.01 dilution, place 1 ml of well mixed raw sample into 99 ml of sterile buffered dilution water. Mix thoroughly by shaking. This bottle will be labeled bottle A.

5. Into each of the next 5 lactose broth tubes place directly 1 ml of the 0.01 dilution, from bottle A.

At this point you have 15 tubes inoculated and can place these three sets of tubes in the incubator; however, your sample specimen may show gas production in all 15 fermentation tubes.

This means your sample was not diluted enough and you have no usable results. To obtain usable results it is recommended that the first time a sample is analysed that 30 tubes having a range of six dilutions be setup. In most cases this will give usable results.

6. To make a 1/1000 or 0.001 dilution add 0.1 ml from the 1/100 dilution bottle (Bottle A) directly into each tube of five more lactose broth tubes.

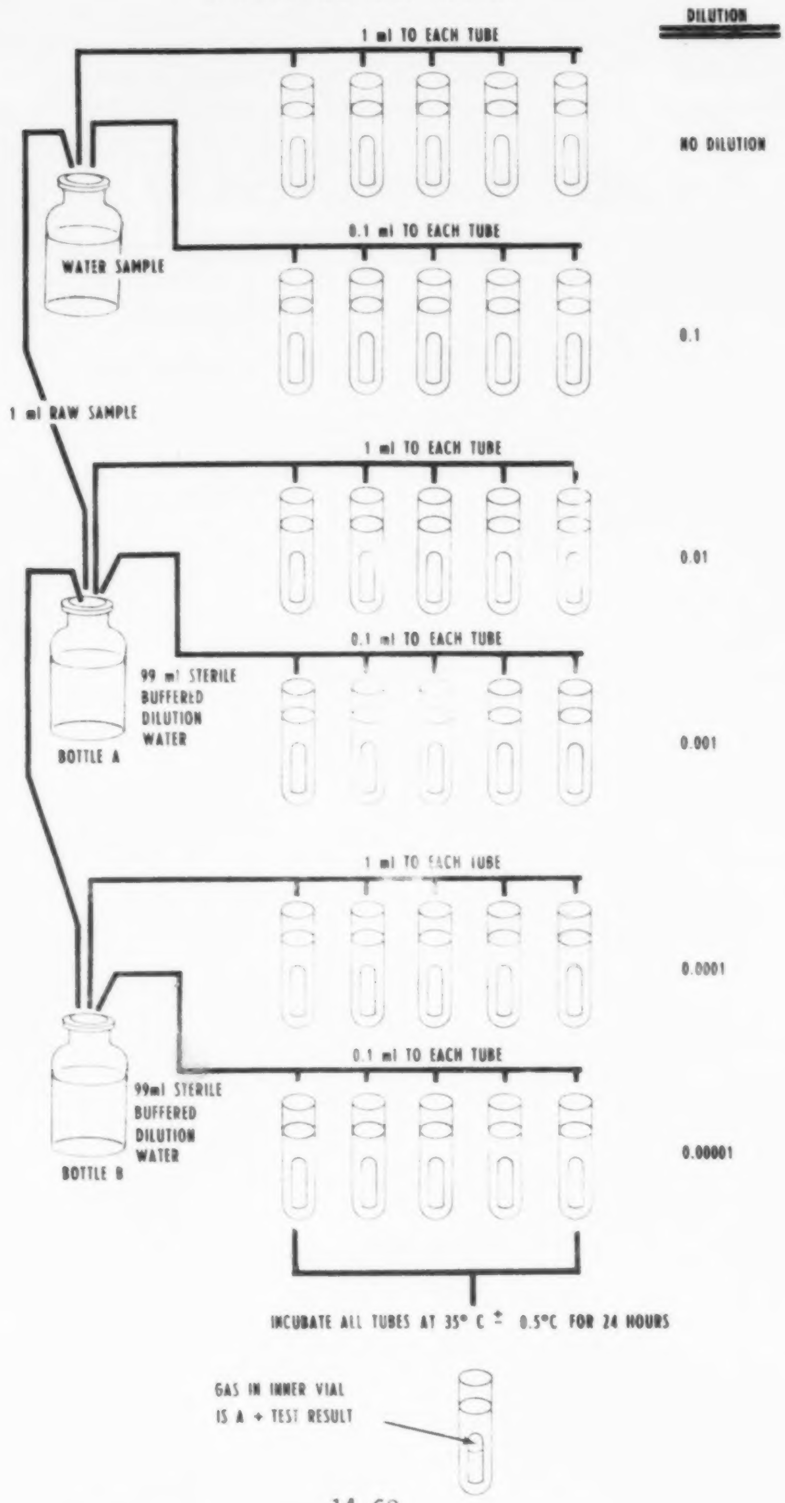
7. To make a 1/10000 or 0.0001 dilution take 1 ml from Bottle A and place this 1 ml into 99 ml of sterile buffered dilution water. Mix diluted sample thoroughly by shaking. This bottle will be called Bottle B.

8. From the 0.0001 dilution (Bottle B) pipette 1.0 ml of sample directly into each tube. Set 5 tubes up with this dilution.

9. To make a 1/100000 or 0.00001 dilution pipette 0.1 ml of sample directly into each tube. Set 5 tubes up with this dilution.

The first time a sample is analysed 30 tubes of lactose broth should be prepared. Once the appropriate dilutions are established that give usable results for determining the MPM Index only 15 tubes need by prepared for subsequent samples to be analysed.

COLIFORM BACTERIA TEST FIG. 14.4


DILUTION
1 ml TO EACH TUBE
NO DILUTION
0.1 ml TO EACH TUBE
WATER SAMPLE
0.1
1 ml RAW SAMPLE
1 ml TO EACH TUBE
0.01
0.1 ml TO EACH TUBE
99 ml STERILE BUFFERED DILUTION WATER
BOTTLE A
0.001
1 ml TO EACH TUBE
0.0001
0.1 ml TO EACH TUBE
99ml STERILE BUFFERED DILUTION WATER
BOTTLE B
0.00001
INCUBATE ALL TUBES AT 35° C ± 0.5°C FOR 24 HOURS
GAS IN INNER VIAL IS A + TEST RESULT

5. 24-Hour Lacotse Broth Presumptive Test

Place all inoculated lactose broth tubes in 35°C ± 0.5°C incubator. After 24 ± 2 hours have elapsed, examine each tube for gas formation in inverted vial (inner tube). Mark + on report form for all tubes that show presence of gas. Mark - for all tubes showing no gas formation. Save all positive tubes for confirmation test. The negative tubes must be reincubated for an additional 24 hours.

6. 48-Hour Lactose Broth Presumptive Test

Record both positive and negative tubes at the end of 48 ± 3 hours. Save all positive tubes for confirmation test.

7. 24-Hour Brilliant Green Bile Confirmation Test

Confirm all presumptive tubes that show gas at 24 or 48 hours. Transfer, with the aid of a sterile 3 mm platinum wire loop, one loop-full of the broth from the lactose tubes showing gas, and inoculate a corresponding tube of BGB (Brilliant Green Bile) broth by mixing the loop of broth in the BGB broth. "Discard" all positive lactose broth tubes after transferring is completed.

Always sterilize inoculation loops and needles in flame immediately before transfer of culture; do not lay loop down or touch it to any nonsterile object before making the transfer. After sterilization in a flame, allow sufficient time for cooling, in the air, to prevent the heat of the loop from killing the bacterial cells being transferred. Sterile wooden applicator sticks also are used to transfer cultures, expecially in the field where a flame is not available for sterilization.

After 24 hours has elapsed, inspect each of the BGB tubes for gas formation. Those with any amount of gas are considered positive and are so recorded on the data sheet. Negative BTB tubes are reincubated for an additional 24 hours.

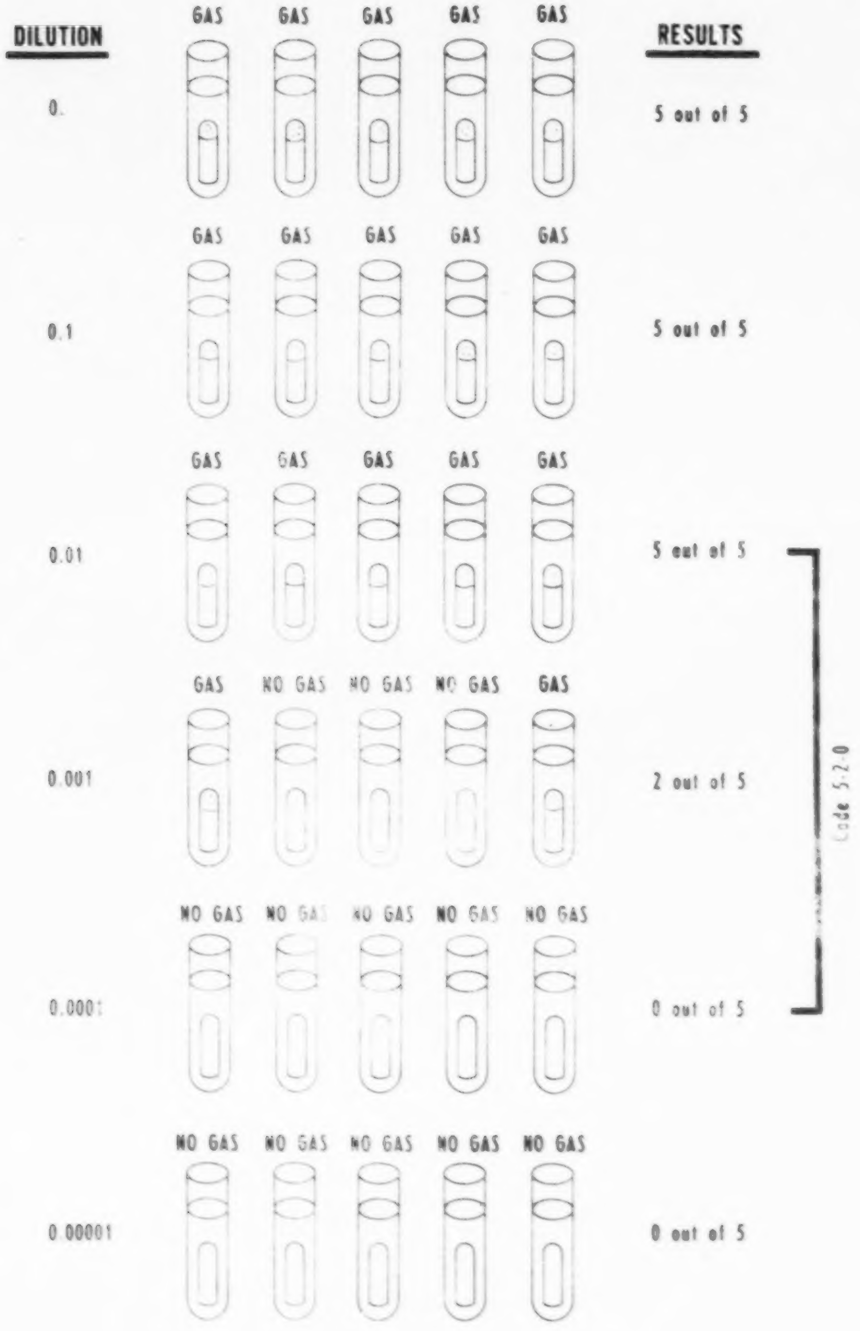
8. 48-Hour Brilliant Green Bile Confirmation Test

1. Examine tubes for gas at the end of the 48 ± 3 hour period. Record both positive and negative tubes.
2. Complete reports by decoding MPN index and recording MPN on work sheets.

9. Methods of calculations of the most probable number

Select the highest dilution with all positive tubes, before a negative tube occurs, plus the next two dilutions, (See Example No. 1).

EXAMPLE NO. 1


DILUTION
RESULTS
GAS GAS GAS GAS GAS
0.
5 out of 5
GAS GAS GAS GAS GAS
0.1
5 out of 5
GAS GAS GAS GAS GAS
0.01
5 out of 5
GAS NO GAS NO GAS NO GAS GAS
0.001
2 out of 5
NO GAS NO GAS NO GAS NO GAS NO GAS
0.0001
0 out of 5
NO GAS NO GAS NO GAS NO GAS NO GAS
0.00001
0 out of 5
Code 5-2-0

From the code 5-2-0 in the MPN Table (Table III) the MPN index is 49. Using the following formula the # of coliform bacteria /100 ml is determined.

$$\text{MPN/100 ml} = \frac{\overset{\text{MPN}}{\text{Index from table}} \times \overset{\text{a constant}}{10}}{\text{Dilution giving } \underline{\text{all}} \ \underline{\text{positive}}\text{, before a negative tube occurs}}$$

$$= \frac{49 \times 10}{0.01} = 49000$$

Example number 2

Dilutions	0	0.1	0.01	0.001	0.0001	0.00001
No. of tubes positive out of 5 tubes set up at each dilution	5	5	5	5	0	0

Our code in example 2 is 5-0-0. Going to the MPN index (Table III) we read the MPN Index of 23. Using the formula as in example number 1

$$\text{MPN/100 ml} = \frac{23 \times 10}{0.001}$$

$$\text{MPN/100 ml} = 230{,}000$$

or

$$\text{MPN} = 230{,}000 \text{ per 100 ml}$$

Example number 3

Dilutions	0	0.1	0.01	0.001	0.0001	0.00001
No. of tubes positive out of 5 tubes set up at each dilution	5	1	0	1	0	0

In example number 3 the code 5-1-0-1 is unreasonable because probability would indicate that the 0.01 dilution should have 1 tube in 5 giving a positive result. In this case it is assumed that the 0.01 dilution should have given 1 tube in 5 as positive and the code 5-1-1 is used to determine the MPN/100 ml.

Using the formula:

$$\text{MPN/100 ml} = \frac{46 \times 10}{0}$$

$$\text{MPN} = 460/100 \text{ ml}$$

TABLE III

MPN INDEX FOR VARIOUS COMBINATIONS OF POSITIVE AND NEGATIVE RESULTS IN A PLANTING SERIES OF FIVE 10-ml, FIVE 1-ml AND FIVE 0.1-ml PORTIONS OF SAMPLE

Number of tubes giving positive reaction out of			MPN Index
Five 10-ml portions	Five 1-ml portions	Five 0.1 ml portions	(organisms per 100 ml)
0	0	0	<2
0	0	1	2
0	0	2	4
0	1	0	2
0	1	1	4
0	1	2	6
0	2	0	4
0	2	1	6
0	3	0	6
1	0	0	2
1	0	1	4
1	0	2	6
1	0	3	8
1	1	0	4
1	1	1	6
1	1	2	8
1	2	0	6
1	2	1	8
1	2	2	10
1	3	0	8
1	3	1	10
1	4	0	11
2	0	1	5
2	0	1	7
2	0	2	9
2	0	3	12
2	1	0	7
2	1	1	9
2	1	2	12
2	2	0	9
2	2	1	12
2	2	2	14

TABLE III (cont'd.)

MPN INDEX FOR VARIOUS COMBINATIONS OF POSITIVE AND NEGATIVE RESULTS IN A PLANTING SERIES OF FIVE 10-ml, FIVE 1-ml AND FIVE 0.1-ml PORTIONS OF SAMPLE

Number of tubes giving positive reaction out of			MPN Index
Five 10-ml portions	Five 1-ml portions	Five 0.1 ml portions	(organisms per 100 ml)
2	3	0	12
2	3	1	14
2	4	0	15
3	0	0	8
3	0	1	11
3	0	2	13
3	1	0	11
3	1	1	14
3	1	2	17
3	1	3	20
3	2	0	14
3	2	1	17
3	2	2	20
3	3	0	17
3	3	1	21
3	4	0	21
3	4	1	24
3	5	0	25
4	0	0	13
4	0	1	17
4	0	2	21
4	0	3	25
4	1	0	17
4	1	1	21
4	1	2	26
4	2	0	22
4	2	1	26
4	2	2	32
4	3	0	27
4	3	1	33
4	3	2	39

TABLE III (cont'd.)

MPN INDEX FOR VARIOUS COMBINATIONS OF POSITIVE AND NEGATIVE RESULTS IN A PLANTING SERIES OF FIVE 10-ml, FIVE 1-ml AND FIVE 0.1-ml PORTIONS OF SAMPLE

Number of tubes giving positive reaction out of			MPN Index
Five 10-ml portions	Five 1-ml portions	Five 0.1 ml portions	(organisms per 100 ml)
4	4	0	34
4	4	1	40
4	5	0	41
4	5	1	48
5	0	0	23
5	0	1	31
5	0	2	43
5	0	3	58
5	0	4	76
5	1	0	33
5	1	1	46
5	1	2	63
5	1	3	84
5	2	0	49
5	2	1	70
5	2	2	94
5	2	3	120
5	2	4	148
5	2	5	177
5	3	0	79
5	3	1	109
5	3	2	141
5	3	3	175
5	3	4	212
5	3	5	253
5	4	0	130
5	4	1	172
5	4	2	221
5	4	3	278
5	4	4	345
5	4	5	426
5	5	0	240
5	5	1	348
5	5	2	542
5	5	3	920
5	5	4	1600
5	5	5	>2400

F. Test for Fecal Coliform Bacteria

1. General Discussion

Many regulatory agencies are measuring the bacteriological quality of water using the fecal coliform test because this test is a more reliable test for indicating the potential presence of pathogenic organisms than is the coliform group of organisms. The procedure described is an elevated temperature test for fecal coliform bacteria.

2. Materials Needed

Equipment required for the tests are the same as those required for the 24-Hour Lactose Broth Presumptive Test, a water bath, and EC Broth.

3. Procedure

1. Run lactose broth or lauryl tryptose broth presumptive test.
2. After 24 hours temporarily retain all gas-positive tubes.
3. Label a tube of EC broth to correspond with each gas-positive tube of broth from presumptive test.
4. Transfer one loop-full of culture from each gas-positive culture in presumptive test to the correspondingly labeled tube of EC broth.
5. Incubate EC broth tubes 24 ± 2 hours at 44.5°C ± 0.2°C in a waterbath with water depth sufficient to come up at least as high as the top of the culture medium in the tubes. Place in waterbath as soon as possible after inoculation and always within 30 minutes after inoculation.
6. After 24 hours remove the rack of EC cultures from the waterbath, shake gently, and record gas production for each tube. Gas in any quantity is a positive test.
7. As soon as results are recorded, discard all tubes. This is a 24-hour test for EC broth inoculations and not a 48-hour test.

8. Transfer any additional 48-hour gas positive tubes from the presumptive test to correspondingly labeled tubes of EC broth. Incubate for 24 ± 2 hours at 44.5°C ± 0.2°C and record results on data sheet.

9. Codify results and determine MPN of fecal coliforms per 100 ml of sample.

G. Membrane Filter Method

1. General Discussion

In addition to the fermentation tube test for coliform bacteria, another test is used for these same bacteria in water analysis. This test uses a cellulose ester filter, called a membrane filter, the pore size of which can be manufactured to close tolerances. Not only can the pore size be made to selectively trap bacteria from water filtered through the membrane, but nutrients can be diffused up through the membrane to grow these bacteria into colonies. These colonies are recognizable as coliform because the nutrients include fuchsin dye which peculiarly colors the colony. Knowing the number of colonies and the volume of water filtered, the operator can then compare the water tested with water quality standards.

2. Materials Needed

1. One sterile membrane filter having a 0.45μ pore size.

2. One sterile 47 mm Petri dish with lid.

3. One sterile funnel and support stand.

4. One sterile pad.

5. One receiving flask (side-arm, 1000 ml).

6. Vacuum pump, trap, suction or vacuum gage, connecting sections of plastic tubing, Glass "T" hose clamp to adjust pressure by-pass.

7. Tweezers, alcohol, Bunsen Burner, grease pencil.

8. Sterile buffered distilled water for rinsing made up in 100-500 ml quantities.

9. M-Endo Media.

10. Sterile pipettes--two 5 ml graduated, one 1 ml for aliquot or one 10 ml for larger aliquot. Quantity of one ml pipettes if dilution of sample is necessary. Also, quantity of dilution water blanks if dilution of sample is necessary.

11. One moist incubator at 35° C temperature. Auxiliary incubator dish with cover.

3. Illustration of Inoculation of Membrane Filter

Fig. I

1. Center membrane filter on filter holder. Handle membrane only on outer 3/16 inch with tweezers sterilized before use in ethyl or methyl alcohol and passed lightly through a flame.

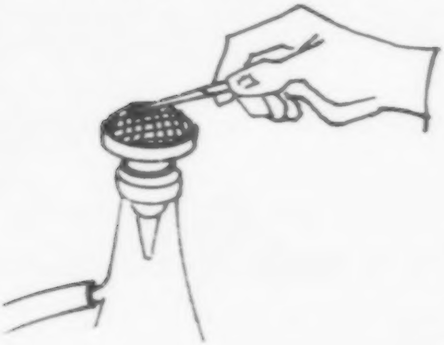


Fig. II

2. Place funnel onto filter holder.

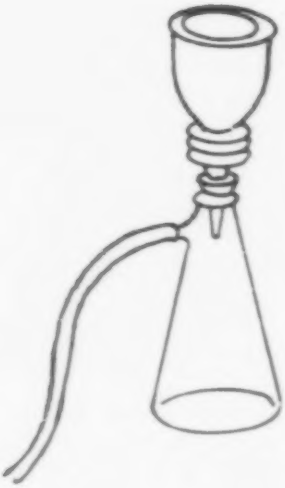
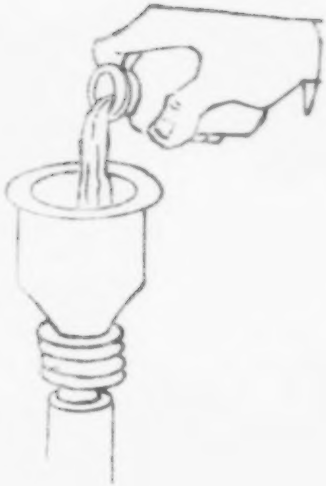
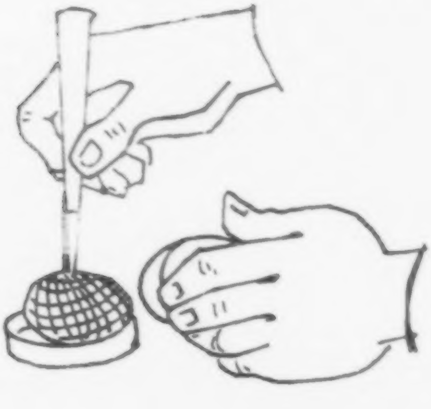


Fig. III



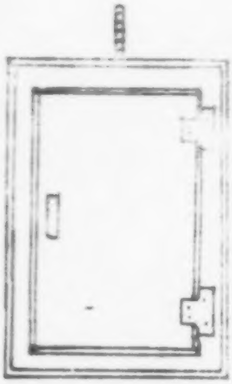
3. Pour or pipette sample aliquot into funnel. Avoid spattering. After suction is applied rinse two times with sterile buffered distilled water.

Fig. IV



4. Remove membrane filter from filter holder with sterile tweezers. Place membrane on pad. Cover with Petri top.

Fig. V



5. Incubate in inverted position for 22 ± 2 hours.

6. Count colonies on membrane.

4. Procedure for Inoculation of Membrane Filter

All filtrations and dilutions of water specimens must be accurate and should be made so that no contaminants from the air, equipment, clothes or fingers reach the specimen either directly or by way of the contaminated pipette.

1. Secure tubing from pump and bypass to receiving flask. Place palm of hand on flask opening and start pump. Adjust suction to ½ atmosphere with hose clamp on pressure bypass. Turn pump switch to OFF.

2. Set sterile filter-support-stand and funnel on receiving flask. Loosen wrapper. Rotate funnel counter-clockwise to disengage pin. Recover with wrapper.

3. Place Petri Dish on bench with lid up. Write identification on lid with grease pencil.

4. Open sterile filter pad package. Light Bunsen burner.

5. Sterilize tweezers by dipping in alcohol and passing quickly through Bensen burner.

6. Center membrane filter on filter stand with tweezers after lifting funnel. Membrane filter with printed grid should show grid uppermost (Fig. 1).

7. Replace funnel and lock against pin (Fig. 11).

8. Add a small amount of the sterile dilution water to funnel. This will help check for leakage and also aid in dispersing small volumes (Fig. 111).

9. Shake sample or diluted sample. Measure proper aliquot[9] with sterile pipette and add to funnel.

10. Now start vacuum pump.

---

[9] Aliquot (AL-li-kwot). Portion of sample.

12. After filtration of entire sample is finished, rinse two times with sterile buffered distilled water, pouring just below inner lip of funnel. Allow each rinse to completely pass through funnel before proceeding to next rinse.

13. When membrane filter appears barely moist, switch pump to OFF.

14. Sterilize tweezers as before.

15. Remove membrane filter with tweezers after first removing funnel as before (Fig. 1).

16. Center membrane filter on pad containing M-Endo medium with a rolling motion to insure water seal. Inspect membrane to insure no captured air bubbles are present. (Fig. IV).

17. Place inverted Petri Dish in incubator for 22 ± 2 hours.

5. Procedure for Counting Membrane Filter Colonies

1. Remove Petri Dish from incubator.

2. Remove lid from Petri Dish.

3. Place Petri Dish with filter under illuminating light. Tilt membrane filter in base of Petri Dish so that green and yellow-green colonies are most apparent. Direct sunlight has too much red to facilitate counting.

4. Count individual colonies utilizing an overhead fluorescent light. The coliform colony is characterized by a "metallic sheen" and only those colonies showing ANY amount of this sheen are considered to be coliforms.

5. Report total number of "coliform colonies" on work sheet. Use the membranes that show from 20 to 80 colonies and do not have more than 200 colonies of all types (including non-sheen or, in other words, non-coliforms).

Example:

A total of 42 colonies grew after filtering 10 ml of the undiluted sample.

$$\text{Bacteria/100 ml} = \frac{\text{No. of colonies counted x 100 ml}}{\text{Volume of sample filtered}}$$

Example: $$= \frac{(42 \text{ colonies})\ (100 \text{ ml})}{(10 \text{ ml})\ (100 \text{ ml})} = \frac{(4.2\ \ (100 \text{ ml})}{100 \text{ ml}} = 420 \text{ per } 100 \text{ ml}.$$

A total of 30 colonies grew after filtering 20 ml of a 1/100 or 0.01 diluted sample.

$$\text{Bacteria/100 ml} = \frac{\text{No. of colonies counted x 100 ml}}{\text{Volume of sample filtered x dilution factor}}$$

$$= \frac{30 \times 100 \text{ ml}}{20 \text{ ml} \times 0.01}$$

$$= 15000 \text{ bacteria/100 ml}$$

QUESTIONS

7.A Why should sodium thiosulfate crystals be added to sample bottles for coliform tests before sterilization?

7.B Steam autoclaves effect sterilization (killing of all organisms) at a relatively low temperature (c ________ °C) within __________ minutes by utilizing moist heat.

7.C Calculate the Most Probable Number (MPN) of coliform group bacteria from the following test results:

Dilutions	0	-1	-2	-3	-4	-5
Readings	5	5	5	1	2	0

7.D How is the number of coliforms estimated by the membrane for filter method?

END OF LESSON 3 of 8 LESSONS

on

Laboratory Procedures and Chemistry

Work the next portion of the discussion and review questions before continuing with Lesson 4.

DISCUSSION AND REVIEW QUESTIONS

(Lesson 3 of 8 Lessons)

Chapter 14. Laboratory Procedures and Chemistry

Name ______________________________ Date ______________

Write the answers to these questions in your notebook. The problem numbering continues from Lesson 2.

8. Why must the clarity test always be run under the same conditions?

9. What is the purpose of the coliform group bacteria test?

10. What does MPN mean?

The following graphical method may be helpful and is added here as an alternate:

PROCEDURE FOR USE OF GRAPH

Step 1: Locate the point on the horizontal axis which corresponds to the coliform value obtained from the laboratory analysis.

Step 2: Draw a vertical line from the point located in Step 1 up to the diagonal line on the graph.

Step 3: Draw a horizontal line from the point on the diagonal line which was located in Step 2 to the vertical axis on the left side of the graph.

Step 4: The point on the vertical axis which was located in Step 3 corresponds to the logarithm of the coliform value.

Step 5: Repeat Steps 1 through 4 for each coliform value which was obtained in the given time period.

Step 6: Sum all of the logarithm values obtained in Step 4.

Step 7: Divide the sum of the logarithms by the number of logarithms summed in Step 6.

Step 8: Locate the point on the vertical axis which corresponds to the value obtained in Step 7.

Step 9: Draw a horizontal line from the point located in Step 8 to the diagonal line on the graph.

Step 10: Draw a vertical line from the point on the diagonal line which was located in Step 9, down to the horizontal axis of the graph.

Step 11: The point on the horizontal axis which was located in Step 10 corresponds to the geometric mean coliform value.

The following graphical method may be helpful and is added here as an alternate:

EXAMPLE OF USE OF GRAPH

Given: The following coliform levels (in numbers per 100 ml) were determined for eight effluent samples collected during a 1-month period: 375, 425, 78, 17, 1098, 8, 9327, and 172.

Find: The monthly geometric mean value of the eight effluent coliform samples.

A. The logarithm of each coliform determination is selected from the graph using Steps 1 through 5 of the procedure.

Sample Coliform Determination	Logarithm of Coliform Determination
1. 375	2.57
2. 425	2.63
3. 78	1.89
4. 17	1.23
5. 1,098	3.04
6. 8	0.90
7. 9,327	3.97
8. 172	2.24
Total =	18.47

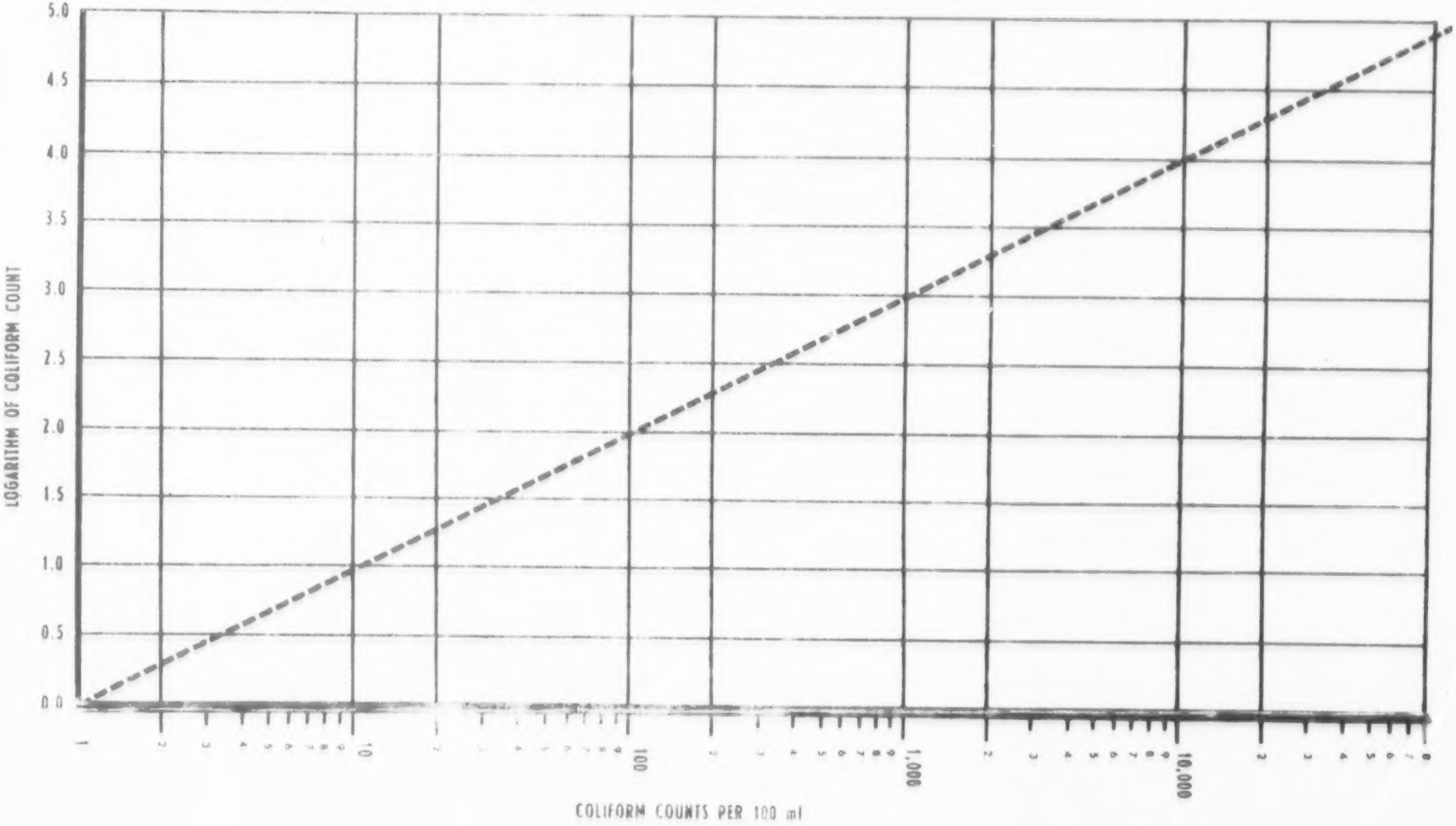
B. The sum of the logarithm determinations is obtained (Step 6 of the procedure).

C. The arithmetic average of the logarithm is obtained (Step 7 of the procedure). Arithmetic average of logarithms =

$$\frac{\text{Total}}{\text{Number of samples}} = \frac{18.47}{8} = 2.31$$

D. The geometric mean value is selected from the graph using Steps 8 through 11 of the procedure. Geometric mean value = 200/100 ml.

GRAPH FOR DETERMINING GEOMETRIC MEANS OF COLIFORM VALUES



3. Dissolved Oxygen or DO and Biochemical Oxygen Demand or BOD

I. IN WATER

A. Discussion

The dissolved oxygen (DO) test is, as the name implies, the testing procedure to determine the amount of oxygen dissolved in samples of water or wastewater. There are various types of tests that can be run to obtain the amount of dissolved oxygen. This procedure is the Sodium Azide Modification of the Winkler Method and is best suited for relatively clean waters. Interfering substances include color, organics, suspended solids, sulfides, chlorine, and ferrous and ferric iron. Nitrites will not interefere with the test if fresh azide is used.

The generalized principle is that iodine will be released in proportion to the amount of dissolved oxygen present in the sample. By using sodium thiosulfate with starch as the indicator, one can titrate the sample and determine the amount of dissolved oxygen.

B. What is Tested?

Sample	Common Range, mg/l
Influent	Usually 0, >1 is very good.
Primary Clar. Effluent	Usually 0, Recirculated from filters > 2 is good.
Secondary Effluent	50% to 95% Saturation, 3 to >8 is good.
Oxidation Ponds	1 to 25+*
Activated Sludge-- Aeration Tank Outlet	>2 desirable

(> means greater than)
(* supersaturated with oxygen)

C. Apparatus

METHOD A (Sodium Azide Modification of Winkler Method)

1. Buret, graduated to 0.1 ml.
2. Three 300 ml glass-stoppered BOD bottles
3. Wide-mouth Erlenmeyer flask, 500 ml.
4. One 10 ml measuring pipette.
5. One 1-liter reagent bottle to collect activated sludge.

METHOD B (DO Probe)

Follow manufacturer's instructions. See Section H for Discussion, Calibration, and Precautions.

D. Reagents

1. Manganous sulfate solution. Dissolve 480 g manganous sulfate crystals ($MnSO_4 \cdot 4H_2O$) in 400 to 600 ml distilled water. Filter through filter paper, then add distilled water to the filtered liquid to make a 1-liter volume.

2. Alkaline iodide-sodium azide solution. Dissolve 500 g sodium hydroxide ($NaOH$) in 500 to 600 ml distilled water; dissolve 150 g potassium iodide (KI) in 200 to 300 ml distilled water in a separate container. Exercise caution. Mix chemicals in pyrex glass bottles using a magnetic stirrer. Add the chemicals to the distilled water slowly and cautiously. Avoid breathing the fumes and body contact with the solution. Heat is produced when the water is added, and the solution is very caustic. Place an inverted beaker over the top of the mixing container and allow the container to cool at room temperature.

   Mix both solutions when they are cool.

   Dissolve 10 g sodium azide (NaN_3) in 40 ml of distilled water. Exercise caution again. This solution is poisonous.

   Add the sodium azide solution with constant stirring to the cooled solution of alkaline iodide; then add distilled water to the mixture to make a 1-liter volume. Sodium azide will decompose in time and is no good after three months.

3. Sulfuric acid. Use concentrated reagent-grade acid (H_2SO_4). Handle carefully, since this material will burn hands and clothes. Rinse affected parts with tap water to prevent injury.

   CAUTION: When working with alkaline azide and sulfuric acid, keep a nearby water faucet running for frequent hand rinsing.

4. 0.0375 N sodium thiosulfate solution. Dissolve exactly 9.308 g sodium thiosulfate crystals ($Na_2S_2O_3 \cdot 5H_2O$) in freshly boiled and cooled water and make up to 1 liter. For preservation, add 0.4 g or 1 pellet of sodium hydroxide ($NaOH$). Solutions of "thio" should be used within two weeks to avoid loss of accuracy due to decomposition of solution.

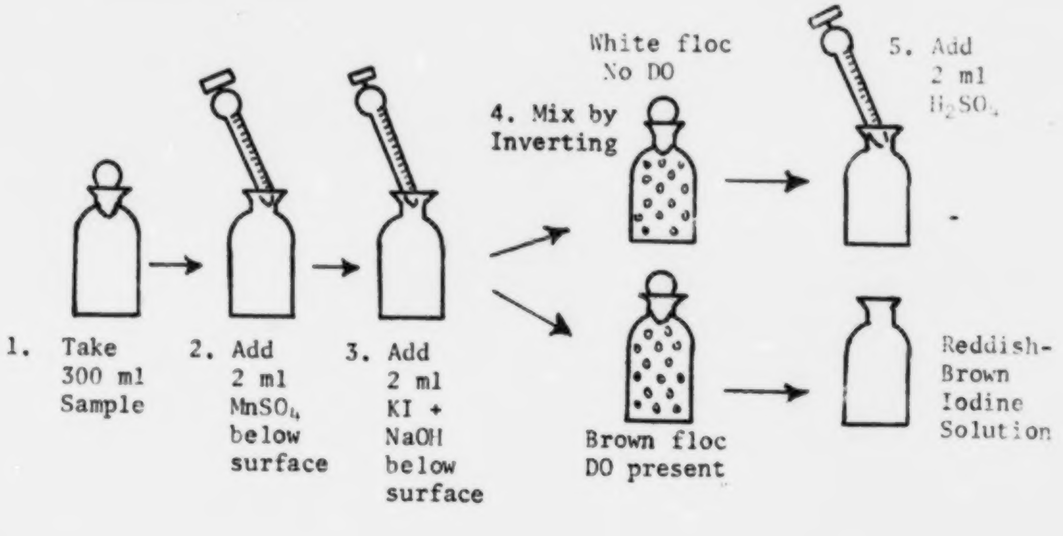
5. Starch solution. Make a thin paste of 6 g of potato starch in a small quantity of distilled water. Pour this paste into one liter of boiling, distilled water, allow to boil for a few minutes, then settle overnight. Remove the clear supernatant and save; discard the rest. For preservation, add two drops toluene ($C_6H_5CH_3$).

6. Copper sulfate solution. Make a 10 percent solution by dissolving 10 grams of copper sulfate in 100 ml of water.

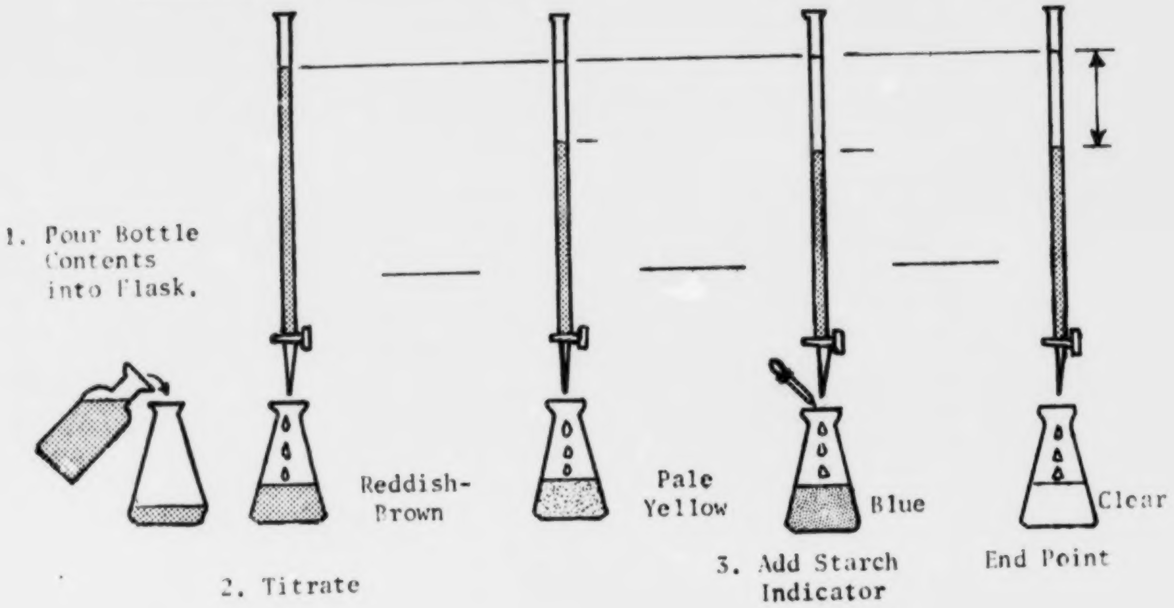
Sodium Azide Modification of the Winkler Method

NOTE: The sodium azide destroys nitrates which will interfere with this test.

E. Outline of Procedure


White floc
No DO
5. Add
2 ml
H_2SO_4
4. Mix by
Inverting
1. Take
300 ml
Sample
2. Add
2 ml
$MnSO_4$
below
surface
3. Add
2 ml
KI +
NaOH
below
surface
Brown floc
DO present
Reddish-
Brown
Iodine
Solution

Titration of Iodine Solution:


1. Pour Bottle Contents into Flask.
2. Titrate
Reddish-Brown
Pale Yellow
3. Add Starch Indicator
Blue
Clear
End Point

PROCEDURE

The reagents are to be added in the quantities, order, and methods as follows:

1. Collect a sample to be tested in 300 ml (BOD) bottle taking special care to avoid aeration of the liquid being collected. Fill bottle completely and add cap.

2. Remove cap and add 2 ml of manganous sulfate solution below surface of the liquid.

3. Add 2 ml of alkaline-iodide-sodium azide solution below the surface of the liquid.

4. Replace the stopper, avoid trapping air bubbles, and shake well by inverting the bottle several times. Repeat this shaking after the floc has settled halfway. Allow the floc to settle halfway a second time.

5. Acidify with 2 ml of concentrated sulfuric acid by allowing the acid to run down the neck of the bottle above the surface of the liquid.

6. Restopper and shake well until the precipitate has dissolved. The solution will then be ready to titrate. Handle the bottle carefully to avoid acid burns.

7. Pour contents of bottle into an Erlenmeyer flask.

8. If the solution is brown in color, titrate with 0.0375 N sodium thiosulfate until the solution is pale yellow color. Add a small quantity of starch indicator and proceed to step 10.

9. If the solution has no brown color, or is only slightly colored, add a small quantity of starch indicator. If no blue color develops, there is zero Dissolved Oxygen. If a blue color does develop, proceed to step 10.

10. Titrate to the first disappearance of the blue color. Record the number of ml of sodium thiosulfate used.

11. The amount of oxygen dissolved in the original solution will be equal to the number of ml of sodium thiosulfate used in the titration provided significant interfering substances are not present.

    mg/l DO = ml sodium thiosulfate

F. Example

The DO titration of a 300 ml sample requires 5.0 ml of 0.0375 N Sodium Thiosulfate. Therefore, the dissolved oxygen concentration in the sample is 5 mg/l.

G. Calculation

You will want to find the percent saturation of DO in the effluent of your secondary plant. The DO is 5.0 mg/l and the temperature is 20°C. At 20°C, 100% DO saturation is 9.2 mg/l.

The dissolved oxygen saturation values are given in Table IV. Note that as the temperature of water increases, the DO saturation value (100% Saturation Column) decreases. Table IV gives 100% DO saturation values for temperatures in °C and °F.

$$\text{DO Saturation, \%} = \frac{\text{DO of Sample, mg/l} \times 100\%}{\text{DO at 100\% Saturation, mg/l}}$$

$$= \frac{5.0 \text{ mg/l}}{9.2 \text{ mg/l}} \times 100\%$$

$$= .54 \times 100\%$$

$$= 54\%$$

$$\begin{array}{r} .54 \\ 9.2 \overline{) 5.0\,0} \\ \underline{4\,6\,0} \\ 4\,00 \\ \underline{3\,68} \\ 32 \end{array}$$

H. DO Probe

1. Discussion

Measurement of the dissolved oxygen (DO) concentration with a probe and electronic readout meter is a satisfactory substitute for the Sodium Azide Modification of the Winkler Method under many circumstances. The probe is recommended when samples contain substances which interfere with the modified Winkler procedure, such as sulfite, thiosulfate, polythionate, mercaptans, free chlorine or hypochlorite, organic substances readily hydrolyzed in alkaline solutions, free iodine, intense color or turbidity, and biological flocs. A continuous record of the dissolved

TABLE IV

EFFECT OF TEMPERATURE ON OXYGEN SATURATION FOR A CHLORIDE CONCENTRATION OF ZERO Mg/l

°C	°F	mg/l DO at Saturation
0	32.0	14.6
1	33.8	14.2
2	35.6	13.8
3	37.4	13.5
4	39.2	13.1
5	41.0	12.8
6	42.8	12.5
7	44.6	12.2
8	46.4	11.9
9	48.2	11.6
10	50.0	11.3
11	51.8	11.1
12	53.6	10.8
13	55.4	10.6
14	57.2	10.4
15	60.0	10.2
16	61.8	10.0
17	63.6	9.7
18	65.4	9.5
19	67.2	9.4
20	68.0	9.2
21	69.8	9.0
22	71.6	8.8
23	73.4	8.7
24	75.2	8.5
25	77.0	8.4

oxygen content of aeration tanks and receiving waters may be obtained using a probe. In determining the BOD of samples, a probe may be used to determine the DO initially and after the five-day incubation period of the blanks and sample dilutions.

2. Procedure

Follow manufacturer's instructions.

3. Calibration

To be assured that the DO probe reading provides the dissolved oxygen content of the sample, the probe must be calibrated. Take a sample that does not contain substances that interfere with either the probe reading or the modified Winkler procedure. Split the sample. Measure the DO in one portion of the sample using the modified Winkler procedure and compare this result with the DO probe reading on the other portion of the sample. Adjust the probe reading to agree with the results from the modified Winkler procedure.

When calibrating the probe in an aeration tank of the activated sludge process, do not attempt to measure the dissolved oxygen in the aerator and then adjust the probe. The biological flocs in the aerator will interfere with the modified Winkler procedure, and the copper sulfate-sulfamic acid procedure is not sufficiently accurate to calibrate the probe. An aeration tank probe may be calibrated by splitting an effluent sample, measuring the DO by the modified Winkler procedure, and comparing results with the probe readings. Always keep the membrane in the tip of the probe from drying because the probe can lose its accuracy until reconditioned.

4. Precautions

1. Periodically check the calibration of the probe.
2. Keep the membrane in the tip of the probe from drying out.
3. Dissolved inorganic salts, such as found in sea water, can influence the readings from a probe.
4. Reactive compounds, such as reactive gases and sulfur compounds, can interfere with the output of a probe.
5. Don't place the probe directly over a diffuser because you want to measure the dissolved oxygen in the water being treated, not the oxygen in the air supply to the aerator.

8. Dissolved Oxygen

II. IN AERATOR

Copper Sulfate-Sulfamic Acid Flocculation, page 413, 12th Edition, 1965, "Standard Methods".

A. Discussion

This modification is used for biological flocs that have high oxygen utilization rates in the activated sludge process, and when a DO probe is not available. It is very important that some oxygen be present in aeration tanks at all times to maintain aerobic conditions.

This test is similar to the regular DO test except that copper sulfate is added to kill oxygen-consuming organisms, and sulfamic acid is added to combat nitrites before the regular DO test is run.

NOTE: If the results indicate a DO of less than 1 mg/l, it is possible that the DO in the aeration tank is ZERO! When the DO in the aeration tank is near zero, considerable DO from the surrounding atmosphere can mix with the sample when it is collected, when the inhibitor is added, while the solids are settling, and when the sample is transferred to a BOD bottle for the DO test. If you use this test, use a deep container and avoid stirring. See article by Hughes and Reynolds JWPCF, Vol. 41, pg. 184, January 1969, for a discussion of the shortcomings of this test.

B. What is Tested?

Sample	Common DO Range, mg/l
Aerator Mixed Liquor	0.1 - 3.0

C. Apparatus

1. One tall bottle, approximately 1000 ml.

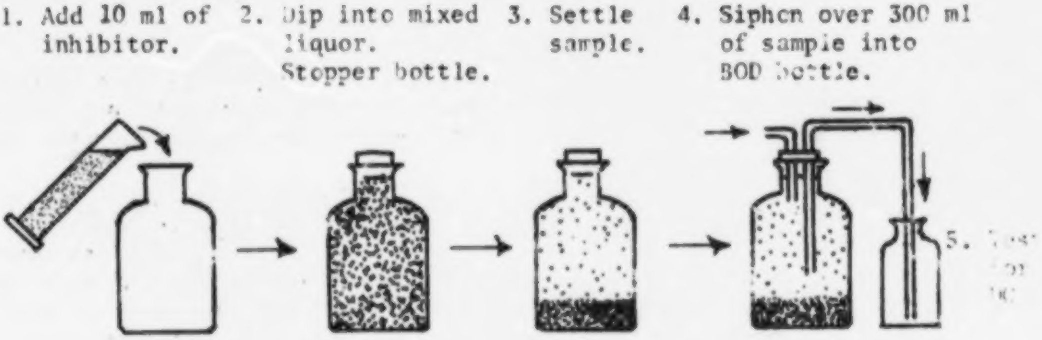
2. Regular DO apparatus.

D. Reagents

1. Copper sulfate-sulfamic acid inhibitor solution. Dissolve 32 g technical grade sulfamic acid (NH_2SO_2OH) without heat in 475 ml distilled water. Dissolve 50 g copper sulfate, $CuSO_4 \cdot 5H_2O$, in 500 ml water. Mix the two solutions together and add 25 ml concentrated acetic acid.

2. Regular DO reagents.

E. Outline of Procedure


1. Add 10 ml of inhibitor.
2. Dip into mixed liquor. Stopper bottle.
3. Settle sample.
4. Siphon over 300 ml of sample into BOD bottle.
5. Test for DO

1. Add at least 10 ml of inhibitor (5 ml copper sulfate and 5 ml sulfamic acid) to any TALL bottle (1-quart milk bottle) with an approximate volume of 1000 ml. Place filling tube near the bottom. An emptying tube is placed approximately 1/4 inch from the top of the bottle cork. Attach bottle to rod or aluminum conduit and lower into aeration tank.

2. Allow bottle to fill and then withdraw.

3. Let stand until clear supernatant liquor can be siphoned into a 300 ml BOD bottle. Do not aerate in transfer.

4. Then run regular DO.

F. and G. Example and Calculations

Same as regular DO test.

QUESTIONS

8.A Calculate the percent dissolved oxygen saturation if the receiving water DO is 7.9 mg/l and the temperature is 10°C.

8.B How would you calibrate the DO probe in an aeration tank?

8.C What are the limitations of the copper sulfate-sulfamic acid procedure for measuring DO in an aeration tank when the DO in the tank is very low?

Biochemical Oxygen Demand or BOD

A. Discussion

The BOD test gives the amount of oxygen used by microorganisms to utilize the substrate (food) in wastewater when placed in a controlled temperature for five days. The DO (dissolved oxygen) is measured at the beginning and recorded. After the 5-day incubation period the DO is again determined. The BOD is then calculated on the basis of the reduction of DO and the size of sample. This test is an estimate of the availability of food in the sample (food for organisms that take up oxygen) expressed in terms of oxygen use. Results of a BOD test indicate the rate of oxidation and provide an indirect estimate of the availability to organisms or concentration of the waste.

Samples are incubated for a standard period of five days because a fraction of the total BOD will be exerted during this period. The ultimate or total BOD is normally never run for plant control. A disadvantage of the BOD test is that the results are not available until five days after the sample was collected.

B. What is Tested?

Sample	Common Range, mg/l
Influent	150 - 400
Primary Effluent	60 - 160
Secondary Effluent	10 - 60
Digester Supernatant	1000 - 4000+
Industrial Wastes	100 - 3000+

C. Apparatus

1. 300 ml BOD bottles with ground glass stoppers
2. Incubator, 20°C
3. Pipettes, 10 ml graduated, 1/32 to 1/16-inch diameter tip
4. Burette and stand
5. Erlenmeyer flask, 500 ml

Reproduced from best available copy.

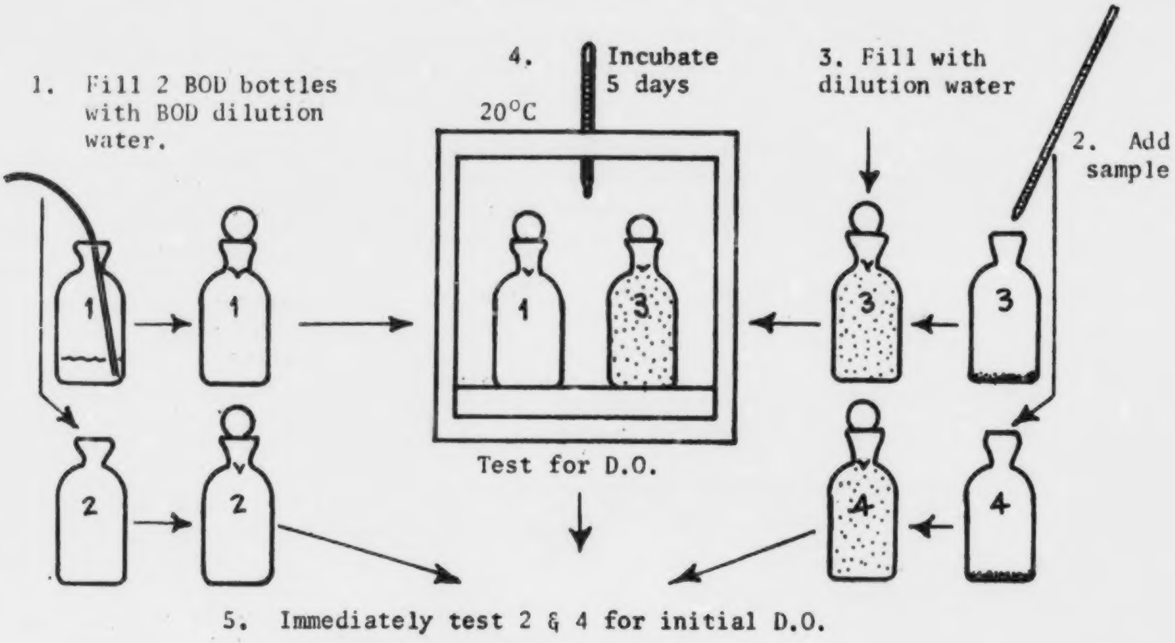
D. Reagents

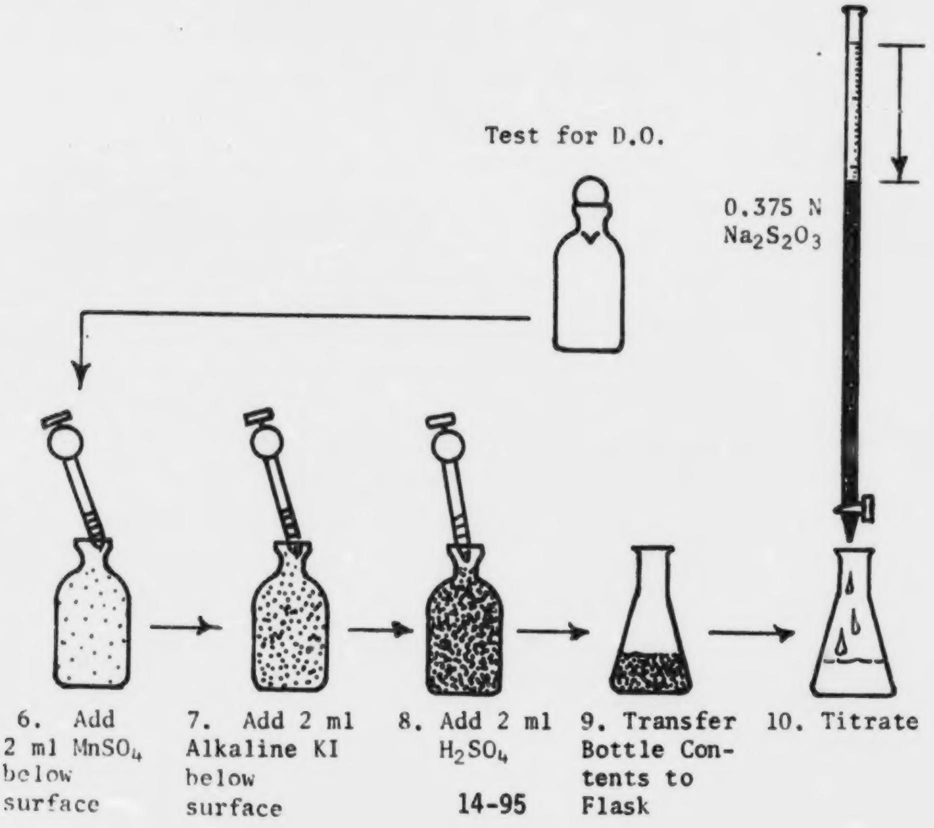
See Section D, page 14-94 under DO portion of this procedure for the preparation of manganous sulfate, alkaline iodide-sodium azide, sulfuric acid, sodium thiosulfate, and starch solutions.

1. Distilled water. Water used for solutions and for preparation of the solution water must be of highest quality. It must contain no copper or decomposable organic matter. Ordinary distilled water for your car's battery is not good enough.
2. Phosphate buffer solution. Dissolve 8.5 g monobasic potassium phosphate (KH_2PO_4), 21.75 g dibasic potassium phosphate (K_2HPO_4), 33.4 g dibasic sodium phosphate crystals ($Na_2HPO_4 \cdot 7H_2O$), and 1.7 g ammonium chloride (NH_4Cl) in distilled water and make up to 1 liter. The pH of this buffer should be 7.3 and should be checked with a pH meter.
3. Magnesium sulfate solution. Dissolve 22.5 g magnesium sulfate crystals ($MgSO_4 \cdot 7H_2O$) in distilled water and make up to 1 liter.
4. Calcium chloride solution. Dissolve 27.5 g anhydrous calcium chloride ($CaCl_2$) in distilled water and make up to 1 liter.
5. Ferric chloride solution. Dissolve 0.25 g ferric chloride ($FeCl_3 \cdot 6H_2O$) in distilled water and make up to 1 liter.
6. Dilution water. Add 1 ml each of phosphate buffer (step 7), magnesium sulfate (step 3), calcium chloride (step 4), and ferric chloride solutions (step 5) for each liter of distilled water. Store at a temperature as close to 20°C as possible for at least 24 hours to allow the water to become stabilized. This water should not show a drop in DO of more than 0.2 mg/l on incubation for five days.

Many plants do not prepare reagents. Small plants and plants that do not run many tests find it quicker and easier to purchase commercially prepared reagents. These reagents may be available in the desired strength or they may consist of dry pillows which are added to the sample, rather than the liquid reagent. Check with your chemical supplier for these reagents.

OUTLINE OF PROCEDURE


1. Fill 2 BOD bottles with BOD dilution water.
4. Incubate 5 days
20°C
3. Fill with dilution water
2. Add sample
1
1
1
3
3
3
2
2
4
4
Test for D.O.
5. Immediately test 2 & 4 for initial D.O.


Test for D.O.
0.375 N $Na_2S_2O_3$
6. Add 2 ml $MnSO_4$ below surface
7. Add 2 ml Alkaline KI below surface
8. Add 2 ml H_2SO_4
9. Transfer Bottle Contents to Flask
10. Titrate

E. Outline of Procedure

The test is made by measuring the oxygen used or depleted during a 5-day period at 20°C by a measured quantity of wastewater sample seeded into a reservoir of dilution water saturated with oxygen. This is compared to an unseeded or blank reservoir of dilution water by subtracting the difference and multiplying by a factor for dilution. See outline on Page 14-107.

PROCEDURE

1. BOD bottles should be of 300 ml capacity with ground glass stoppers and numbers. To clean the bottles, carefully rinse with tap water followed by distilled water.

2. Fill two bottles completely with dilution water and insert the stopper tightly so that no air is trapped beneath the stopper. Siphon dilution water from its container when filling BOD bottles.

3. Set up one or more dilutions of the sample to cover the estimated range of BOD values. From the estimated BOD, calculate the volume of raw sample to be added to the BOD bottle based on the fact that:

   The most valid DO depletion is 4 mg/l. Therefore,

$$\text{ml of sample added per 300 ml} = \frac{(4\ \text{mg/l})\ (300\ \text{ml})}{\text{Estimated BOD, mg/l}} = \frac{1200}{\text{Estimated BOD, mg/l}}$$

   Examples:

   a. Estimated BOD = 400 mg/l

$$\text{ml of sample added to BOD bottle} = \frac{1200}{400} = 3\ \text{ml}$$

b. Estimated BOD = 200 mg/l: use 6 ml
100 mg/l: use 12 ml
20 mg/l: use 60 ml

When the BOD is unknown, select more than one sample size. For example, place several samples--1 ml, 3 ml, 6 ml, and 12 ml--into four BOD bottles.

For samples with very high BOD values, it may be difficult to accurately measure small volumes or to get a truly representative sample. In such a case, initial dilution should first be made on the sample. A dilution of 1:10 is convenient.

4. To perform the BOD test, first fill two BOD bottles with BOD dilution water. Nos. (1) and (2) in illustration, Page 14-107
5. Next, for each sample to be tested, carefully measure out the two portions of sample and place them into two new BOD bottles, Nos. (3) and (4). Add dilution water until the bottles are completely filled. Insert the stoppers. Avoid entrapping air bubbles. Be sure that there are water seals on the stoppers.
6. On bottles (2) and (4) immediately determine the initial dissolved oxygen.
7. Incubate the remaining dilution water blank and diluted sample at 20°C for five days. These are bottles (1) and (3).
8. At the end of exactly five days (± 3 hours), test bottles (1) and (3) for their dissolved oxygen by using the sodium azide modification of the Winkler method or a DO probe. At the end of five days, the oxygen content should be at least 1 mg/l. Also, a depletion of 2 mg/l or more is desirable. Bottles (1) and (2) are only used to check the dilution water quality. Their difference should be less than 0.2 mg/l if the quality is good and free of impurities.

F. Precautions

Since this is a bioassay (BUY-o-ass-SAY), that is, living organisms are used for the test, environmental conditions must be quite exact.

1. The temperature of the incubator must be at 20°C. Other temperatures will change the rate of oxygen used.
2. The dilution water should be made according to Standard Methods for the most favorable growth rate of the bacteria. This water must be free of copper which is often present when copper stills are used by commercial dealers. Use all glass or stainless steel stills.
3. The wastewater must also be free of toxic wastes, such as hexavalent chromium.
4. If you use a cleaning solution to wash BOD bottles, be sure to rinse the bottles several times. Cleaning agents are toxic and if any residue remains in a BOD bottle, a BOD test could be ruined.
5. Wastewater normally contains an ample supply of seed bacteria; therefore seeding is usually not necessary.

G. Chlorinated Samples

It is very difficult to obtain reliable and reproducible results from the BOD test, and a chlorinated sample is even more difficult. For this reason, samples for BOD tests should be collected before chlorination.

H. Example

BOD Bottle Volume = 300 ml

Sample Volume = 15 ml

Initial DO of Diluted Sample = 8.0 mg/l

DO of Sample and Dilution After 5-day Incubation = 4.0 mg/l

I. Calculations

$$\text{BOD, mg/l} = \left(\begin{array}{l}\text{Initial DO of}\\ \text{Diluted Sam-}\\ \text{ple, mg/l}\end{array} - \begin{array}{l}\text{DO of Diluted}\\ \text{Sample After}\\ \text{5-Day Incuba-}\\ \text{tion, mg/l}\end{array}\right)\left(\frac{\text{BOD Bottle Vol., ml}}{\text{Sample Volume, ml}}\right)$$

$$= (8.0\ \text{mg/l} - 4.0\ \text{mg/l})\left(\frac{300\ \text{ml}}{15\ \text{ml}}\right)$$

$$= \frac{(4.0)\ (300)}{15}$$

$$= 80\ \text{mg/l}$$

For acceptable results, the percent depletion of oxygen in the BOD test should range from 30% to 80% depletion.

$$\%\ \text{Depletion} = \frac{\left(\begin{array}{l}\text{DO of Diluted Sample, mg/l}\\ \quad -\ \text{DO After 5 Days, mg/l}\end{array}\right)}{\text{DO of Diluted Sample, mg/l}} \times 100\%$$

$$= \frac{(8.0\ \text{mg/l} - 4.0\ \text{mg/l})}{8.0\ \text{mg/l}} \times 100\%$$

$$= \frac{4}{8} \times 100\%$$

$$= 50\%$$

When a sample requires a large volume in the BOD test and a small amount of dilution water, or if a sample has a high DO (plant or pond effluent), the initial DO of the mixture may be determined as follows.

Example: BOD Bottle Volume = 300 ml
Sample Volume = 60 ml
Sample DO = 2.0 mg/l
DO of Dilution Water = 8.0 mg/l
DO of Sample and Dilution After 5-Day Incubation = 4.0 mg/l

$$\begin{array}{l}\text{DO of Initial}\\ \text{Mixture of}\\ \text{Dilution Water}\\ \text{and Sample, mg/l}\end{array} = \frac{\text{ml of Sample x DO of Sample + ml of Dilution } H_2O \text{ x DO of Dilution } H_2O}{\text{BOD Bottle Volume}}$$

$$\text{BOD, mg/l} = \frac{60 \text{ ml x } 2.0 \text{ mg/l} + 240 \text{ ml x } 8.0 \text{ mg/l}}{300 \text{ ml}}$$

$$= \frac{120 + 1920}{300} \qquad \begin{array}{r} 6.8 \\ 300\overline{)\,2040.0} \\ \underline{1800} \\ 240.0 \\ 240.0 \end{array}$$

$$= 6.8 \text{ mg/l}$$

$$= \left(\begin{array}{l}\text{DO of}\\ \text{Diluted}\\ \text{Sample,}\\ \text{mg/l}\end{array} - \begin{array}{l}\text{DO After}\\ \text{5 Days,}\\ \text{mg/l}\end{array}\right) \left(\frac{\text{BOD Bottle Vol., ml}}{\text{Sample Vol., ml}}\right)$$

$$= (6.8 \text{ mg/l} - 4.0 \text{ mg/l}) \left(\frac{300 \text{ ml}}{60 \text{ ml}}\right)$$

$$= (2.8)\left(\frac{300}{60}\right) \qquad \begin{array}{r} 5 \\ 60\overline{)\,300} \end{array} \qquad \begin{array}{r} 2.8 \\ \underline{5} \\ 14.0 \end{array}$$

$$= 14.0 \text{ mg/l}$$

NOTES

1. On effluent samples where the DO is run on the sample and the blue bounces back on the end point titration, this indicates nitrite interference and can cause the BOD to be higher than actual by as much as 10% to 15% of the answer. This fact should be considered in interpreting your results. The end point also may waver because of decomposition of azide in an old reagent or resuspension of sample solids. To correct a wavering end point, try preparing a new alkaline-azide solution or more of the old solution should be used because it may be decomposing.

2. Researchers and equipment manufacturers are continually striving to develop quicker and easier tests to measure BOD. If you find a test procedure that provides you with an effective operational control test, use it. Be sure to check with your regulatory agencies for the procedures they require you to use in your effluent monitoring program.

QUESTIONS

8.D How would you determine the amount of organic material in wastewater?

8.E How would you prepare dilutions to measure the BOD of cannery waste having an expected BOD of 2000 mg/l?

8.F What is the BOD of a sample of wastewater if a 2 ml sample in a 300 ml BOD bottle had an initial DO of 7.5 mg/l and a final DO of 3.9 mg/l?

8.G Why should samples for the BOD be collected before chlorination?

8.H Why should opened bottles of "Thio" be used or restandardized within two weeks?

END OF LESSON 4 OF 8 LESSONS

on

Laboratory Procedures and Chemistry

Work the next portion of the discussion and review questions before continuing with Lesson 5.

DISCUSSION AND REVIEW QUESTIONS

(Lesson 4 of 8 Lessons)

Chapter 14. Laboratory Procedures and Chemistry

Name ______________________________ Date ______________

Write the answers to these questions in your notebook. The problem numbering continues from Lesson 3.

11. What is the formula for calculating the percent saturation of DO?

12. What precautions should be exercised when using a DO probe?

13. What is a blank, as referred to in laboratory procedures?

14. What are some of the disadvantages of the BOD test?

15. What precautions should be taken when running a BOD test?

16. Calculate the BOD of a 5 ml sample if the initial DO of the diluted sample was 7.5 mg/l and the DO of diluted sample after 5-day incubation was 3.0 mg/l?

CHAPTER 14. LABORATORY PROCEDURES AND CHEMISTRY

(Lesson 5 of 8 Lessons)

9. Hydrogen Sulfide (H_2S)

I. IN ATMOSPHERE

A. Discussion

The rate of concrete corrosion is often directly related to the rate of H_2S production or amount of H_2S in the atmosphere. This test deals with the time it takes a paper tape or unglazed tile to turn black. It is a qualitative measurement of the H_2S present in the sewer atmosphere. H_2S is recognized by its characteristic odor of rotten eggs.

B. What is Tested?

Sample	Common Range
Atmosphere in sewers, outlets from force mains, wet pits, pumping stations, and influent areas to treatment plants.	Not black in 24 hours = Good, 24+ hr
	Black in less than 1 hour = Bad, < 1 hr

C. Apparatus

Lead acetate paper or unglazed tile soaked in lead acetate.

D. Reagents

Saturated lead acetate solution.

E. Procedure

1. Obtain pieces of unglazed tile or use lead acetate paper. Cut tile with hacksaw into ½ inch strips.

2. Soak strips in tile in lead acetate solution.

3. Dry tile in drying oven or air dry.
4. An open manhole or any point where wastewater is exposed to the atmosphere is a good test site. Drive a nail between metal crown

ring of manhole, concrete, or other convenient place. Tie paper or tile with cotton string to nail and then replace it and return in half an hour or less. If tile is not black or substantially colored, return periodically until black. If H_2S is present as indicated by a color change, then measure flow, temperature, pH, and BOD for further evaluation of problem.

II. IN WASTEWATER

A. Discussion

In sewers, when there is no longer any dissolved oxygen, H_2S tests are run to determine the rate of H_2S increase as the wastewater travels to a pumping station or treatment plant. If the wastewater is exposed to the atmosphere, H_2S will be released and a typical rotten egg odor will be detected. Anaerobic bacteria found in wastewater can liberate H_2S from the solids. When the gas leaves the wastewater stream and comes in contact with moisture and oxygen, sulfuric acid is formed which is very corrosive to concrete. Not all odors in wastewater are from H_2S, and there is no correlation between H_2S and other odors. The total H_2S procedure is good up to 18 mg/l, and higher concentrations must be diluted before testing. H_2S production can be controlled by up-sewer aeration which reduces H_2S formation and also stabilizes the wastewater in the collection system.

B. What is Tested?

Sample Wastewater From the Following Locations	Possible Results, mg/l Good	Bad
Sewers	.1	1
Outlets from force mains	.1	1
Wet pits, pumping stations	.1	.5
Influents to treatment plants	Preferably 0	.5

All of the above locations should be sampled, if pertinent, when using up-stream aeration to control H_2S.

C. Apparatus

1. One LaMotte-Pomeroy Sulfide Testing Kit to test:

   a. Total Sulfides

   b. Dissolved Sulfides

   c. Hydrogen Sulfide in solution

   Obtain from LaMotte Chemical Products Company. Order by Code #4630, $27.50, FOB, Chestertown, Maryland 21620.

2. One LaMotte-Pomeroy Accessory Hydrogen Sulfide Kit for testing H_2S in air and gases (not essential). Obtain from LaMotte Chemical Products Company. Order by Code #4632, $22.00, FOB, Chestertown, Maryland 21620.

D. Reagents

The instructions are in the kit.

E. Procedure

The instructions are in the kit.

Note: No EPA evaluation of test kit was available at time this manual was prepared.

F. Example

The instructions are in the kit.

G. Calculations

The instructions are in the kit.

QUESTION

9.A Why would you measure the H_2S concentration:

1. In wastewater?
2. In the atmosphere?

10. pH

A. Discussion

The intensity of the alkaline or acid strength of water is expressed by its pH.

Mathematically, pH is the logarithm of the reciprocal of the hydrogen ion concentration, or the negative logarithm of the hydrogen ion concentration.

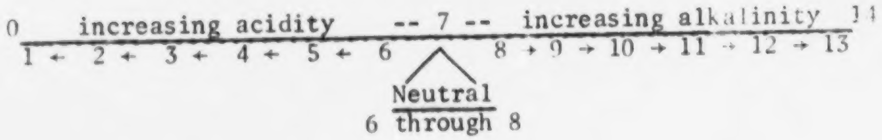
$$\mathrm{pH} = \log \frac{1}{(\mathrm{H}^+)} = -\log (\mathrm{H}^+)$$

For Example

If a wastewater has a pH of 1, then the hydrogen ion concentration $(\mathrm{H}^+) = 10^{-1} = 0.1$.

If pH = 7, then $(\mathrm{H}^+) = 10^{-7} = 0.0000001$.

pH Scale



In a solution, both hydrogen ions (H^+) and the hydroxyl ions (OH^-) are always present. At a pH of 7, the concentration of both hydrogen and hydroxyl ions equals 10^{-7} moles per liter. When the pH is less than 7, the concentration of hydrogen ions is greater than the hydroxyl ions. The hydroxyl ion concentration is greater than the hydrogen ions in solutions with a pH greater than 7.

The pH test indicates whether a treatment process may continue to function properly at the pH measured. Each process in the plant has its own favorable range of pH which must be checked routinely. Generally a pH value from 6 to 8 is acceptable for best organism activity.

The paper tape colorimetric comparison method is explained in this section. This is not considered a "Standard Method" but will give a rough indication of the pH. Most wastewater contains many dissolved solids and buffers which tend to minimize pH changes.

There are many ranges of pH tapes available. Normally a range of 5 to 8 will cover the inplant control testing.

B. What is Tested?

Wastewater	Common Range
Influent or Raw Wastewater (domestic)	6.8 to 8.0
Raw Sludge (domestic)	5.6 to 7.0
Digester Recirculated Sludge or Supernatant	6.8 to 7.2
Plant Effluent Depending on Type of Treatment	6.0 to 8.0

C. Minimum Apparatus List

1. pH Meter.

or 2. Three rolls of paper tapes (range 5 to 8).

or 3. Colorimetric set (range 6.8 to 8.4)--permanent glass which can be used with chlorine comparator or liquid color tubes that are less stable.

D. Reagents

(to be used with corresponding apparatus listed under Section C)

1. Buffer tablets of various pH values. Distilled water.

2. None.

3. Brom thyml blue (for pH 6.2 to 7.6).
Phenol red (for 6.4 to 8.0).

E. Procedures

Use the same samples used for the other tests.

METHOD A (pH Meter)

Procedure

1. Due to the differences between the various makes and models of pH meters commercially available, specific instructions cannot be provided for the correct operation of all instruments. In each case, follow the manufacturer's instructions for preparing the electrodes and operating the instrument.
2. Standardize the instrument against a buffer solution with a pH approaching that of the sample.
3. Rinse electrodes thoroughly with distilled water after removal from buffer solution.
4. Place electrodes in sample and measure pH.
5. Remove electrodes from sample, rinse thoroughly with distilled water.
6. Immerse electrode ends in beaker of pH 7 buffer solution.
7. Shut off meter.

Precautions

1. To avoid faulty instrument calibration, prepare fresh buffer solutions as needed, once per week, from commercially available buffer tablets.
2. pH meter, buffer solution, and samples should all be at the same temperature (constant) because temperature variations will give erroneous results.
3. Watch for erratic results arising from electrodes, faulty connections, or fouling of electrodes with oily or precipitated matter.

METHOD B (Paper Tape)

Procedure

1. Measure pH directly in tank or immediately after collecting sample.

2. Tear off tape 1½ to 2" long. Dip half of tape in tank or sample and quickly read results.

3. Remove tape and compare color with colors on package, and record pH on Laboratory Work Sheet in proper column from which the sample came. For example, if the sample came from the plant influent and the color of the portion of the tape wetted by the sample matches a color on the package indicating a pH of 7.2, then record 7.2 on Laboratory Work Sheet in the influent column on the pH row. (See Fig. 14.2 second page of work sheet).

This procedure applies to liquids that have solids which separate (settle or float) easily.

METHOD B (Paper Tape-High Solids Conc. in Sample)

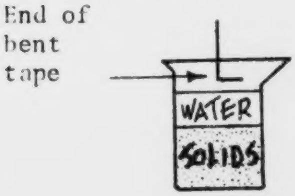
Procedure

The following procedure is for samples containing higher solid concentrations such as found in the raw sludge, digester recirculated sludge, digester supernatant, and digested sludge samples.

1. Obtain representative samples and identify them.

2. Allow samples to stand until some of the solids have settled and water is visible above the solids. Sufficient water should be above the solids to allow the tape to be dipped in the sample and not discolored by the solids.

3. Bend the tape by making a sharp crease ½" from end. Very carefully allow tape to touch liquid surface.



4. Remove tape from liquid surface and compare the color with pH color standard on the package. Record on Laboratory Work Sheet.

METHOD C (Colorimetric Comparitor)

Procedure

1. Fill the three tubes or two rectangular bottles provided with the comparitor unit to the indicator line with a portion of the sample being tested.

2. Add the recommended amount of indicator solution.

3. Place the tubes in the comparitor in such a way that the color standards are opposite the tubes not containing the indicator solution.

4. Compare the colors by rotating the comparitor disk or changing the standard color solution vials. Read the pH of the indicator having the color closest to the color of the sample. Record results on Laboratory Work Sheet.

5. Thoroughly wash and dry sample tubes when test is completed and before returning tubes to comparitor unit for storage.

F. Precautions

1. Collect fresh samples and test immediately. The pH of a sample can change rapidly due to loss of CO_2 and biological activity. A fresh effluent sample could have a pH of 6.5 and after standing overnight the pH could be 8.0.

2. Always measure aerator pH directly in the aerator.

3. The pH of a composite sample will not accurately describe pH conditions in your plant. A ten-minute slug of a highly acid waste can upset plant performance for a day or longer, but you may not notice it in a composite sample. Measure pH in place, frequently and quickly, for best description of environment encountered by organisms in treatment processes.

QUESTIONS

10.A How would you measure the pH by the paper tape colorimetric comparison method for:

1. Plant influent?

2. Raw sludge?

10.B What precautions should be exercised when using a pH meter?

I. SETTLEABILITY

A. Discussion

This test is run on mixed liquor or return sludge and plotted on attached graph (Fig. 14.5). All pertinent information is filled in for process control of aerators.

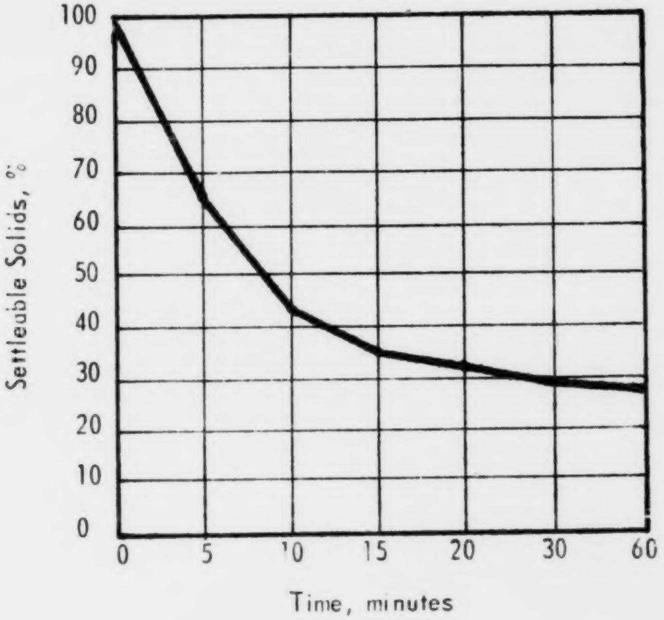


Fig. 14.5 Settleability of activated sludge solids

Settleability is important in determining the ability of the solids to separate from the liquid in the final clarifier. The activated sludge solids should be returned to the aeration tank, and the quality of the effluent is dependent upon the absence of solids flowing over the effluent weir.

The suspended solids should be run on the same sample of mixed liquor that the settleability test is run. This will allow you to calculate the Sludge Volume Index (SVI) or the Sludge Density Index (SDI) which are explained in other sections.

The 2000 ml graduate that is filled with mixed liquor in the settleability test is supposed to indicate what will happen to the mixed liquor in the final clarifier--the rate of sludge settling, turbidity, color, and volume of sludge at the end of 60 minutes.

B. What is Tested?

Sample	Working Range
Mixed Liquor or Return Sludge	Depends on desirable mixed liquor concentration

C. Apparatus

2000 ml graduated cylinder.[10]

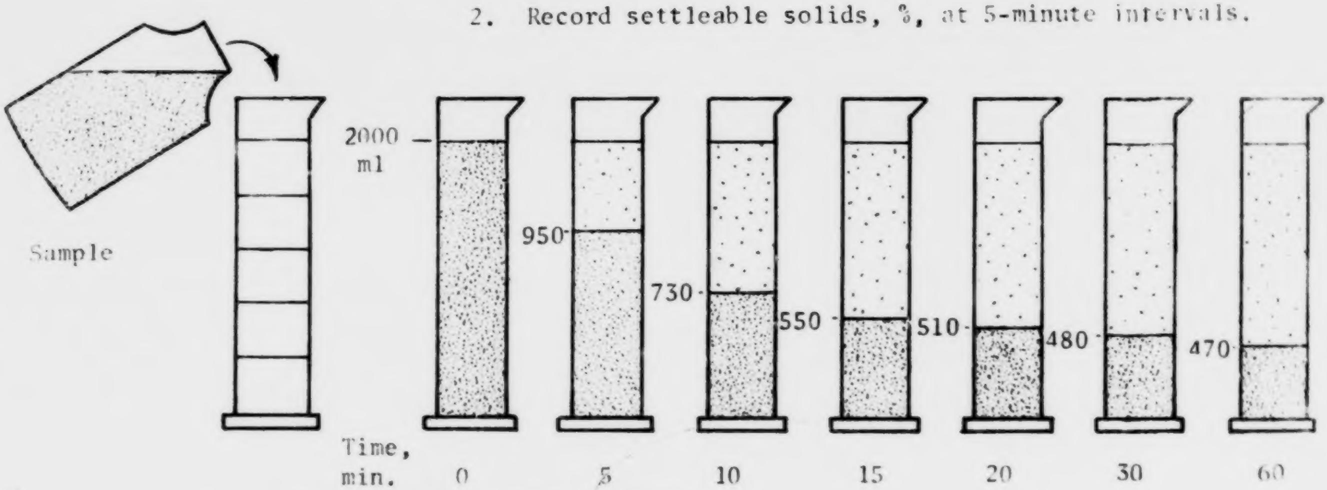
D. Reagents

None.

E. Procedure

1. Mix sample and pour into 2000 ml graduate.

2. Record settleable solids, %, at 5-minute intervals.



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[10] Mallory Direct Reading Settleometer (a 2 liter graduated cylinder approximately 5 inches in diameter and 7 inches high). Obtain from Scientific Glass Apparatus Co., Inc., 735 Broad Street, Bloomfield, New Jersey. Catalog No. JS-1035. Price $16.50 each.

1. Collect a sample of mixed liquor or return sludge.

2. Carefully mix sample and pour into 2000 ml graduate. Vigorous shaking or mixing tends to break up floc and produces slower settling or poorer separation.

3. Record settleable solids, %, at regular intervals.

F. Example and Calculation

The percent settling rate can be compared for the various days of the week and with other measurements--suspended solids, SVI, percent sludge solids returned, aeration rate, and plant inflow. A very slow settling mixed liquor usually requires air and solids adjustment to encourage increased stabilization during aeration. A very rapidly settling mixed liquor usually gives poor effluent clarification.

II. SLUDGE VOLUME INDEX (SVI)

A. Discussion

The Sludge Volume Index (SVI) is used to indicate the condition of sludge (aeration solids or suspended solids) for settleability in a secondary or final clarifier. The SVI is the volume in ml occupied by one gram of mixed liquor suspended solids after 30 minutes of settling. It is a useful test to indicate changes in sludge characteristics. The proper SVI range for your plant is determined at the time your final effluent is in the best condition regarding solids and BOD removals and clarity.

B. What is Tested?

Sample	Preferable Range, SVI
Aerator Solids or Suspended Solids	100 - 250

C. Apparatus

See 11. Settleability of Activated Sludge Solids, Part I, Settleability, and 16. Suspended Solids.

D. Reagents

None.

E. Procedure

See Section 11, I, on Settleability, and 16, Suspended Solids.

F. Example

30-minute settleable solids test = 360 ml or 18%.

Mixed liquor suspended solids = 1500 mg/l.

G. Calculations

$$\begin{aligned}\text{Sludge Volume Index, SVI} &= \frac{\%\ \text{Settleable Solids} \times 10{,}000}{\text{Mixed Liquor Suspended Solids, mg/l}} \\ &= \frac{18 \times 10{,}0\cancel{0}\cancel{0}}{15\cancel{0}\cancel{0}} \\ &= \frac{1800}{15} \\ &= 120\end{aligned}$$

$$\begin{array}{r} 120 \\ 15\overline{)\,1800} \\ \underline{15} \\ 30 \\ \underline{30} \\ 0 \end{array}$$

III. SLUDGE DENSITY INDEX (SDI)

A. Discussion

The Sludge Density Index (SDI) is used in a way similar to the SVI to indicate the settleability of a sludge in a secondary clarifier or effluent. The calculation of the SDI requires the same information as the SVI test.

$$\text{SDI} = \frac{\text{mg/l of suspended solids in mixed liquor}}{\text{ml/l of settled mixed liquor solids} \times 10}$$

or

$$\text{SDI} = 100/\text{SVI}$$

B. What is Tested?

Sample	Preferable Range, SDI
Aerator Solids or Suspended Solids	0.4 - 1.0

C. through G.

These items are not included because of their similarity to the SVI test.

QUESTIONS

II.A Why should you run settleability tests on mixed liquor?

II.B What is the Sludge Volume Index (SVI)?

II.C Why is the SVI test run?

II.D What is the relationship between the Sludge Density Index (SDI) and SVI?

END OF LESSON 5 OF 8 LESSONS

on

Laboratory Procedures and Chemistry

Work the next portion of the discussion and review questions before continuing with Lesson 6.

DISCUSSION AND REVIEW QUESTIONS

(Lesson 5 of 8 Lessons)

Chapter 14. Laboratory Procedures and Chemistry

Name ______________________________ Date __________

Write the answers to these questions in your notebook. The problem numbering continues from Lesson 4.

17. Hydrogen sulfide is measured because it causes ______________
____.

18. What factors promote H_2S production in sewers?

19. The pH scale runs from _____ to ______, with 7 being neutral.

20. Calculate the SVI if the mixed liquor suspended solids are 2000 mg/l and the 30-minute settleable solids test is 500 ml or 25%.

21. Calculate the SDI if the SVI is 125.

12. Settleable Solids

A. Discussion

The settleable solids test is the volume of settleable solids in one liter of sample that will settle to the bottom of an Imhoff cone during a specific time period. The test is an indication of the volume of solids removed by sedimentation in sedimentation tanks, clarifiers, or ponds. The results are read directly in milliliters from the Imhoff cone.

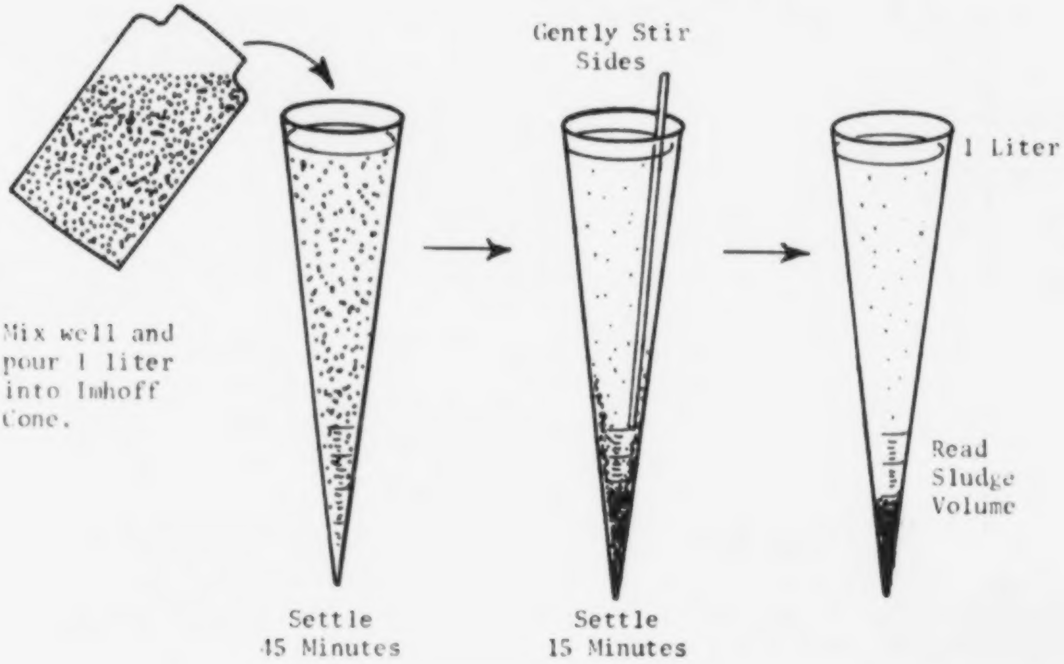
B. What is Tested?

Sample	Common Ranges Found
Influent	12 ml/l medium wastewater 20 ml/l strong wastewater 8 ml/l weak wastewater
Primary Effluent	0.1 ml/l - 3 ml/l
Secondary Effluent	Trace --0.5 ml/l Over .5 ml/l poor

C. Apparatus

1. Imhoff Cones.
2. Rack for holding Imhoff Cones.
3. Glass stirring rod, or wire.

D. Outline of Procedure


Mix well and pour 1 liter into Imhoff Cone.
Settle 45 Minutes
Gently Stir Sides
Settle 15 Minutes
1 Liter
Read Sludge Volume

PROCEDURE

1. Thoroughly mix the wastewater sample by shaking and immediately fill an Imhoff cone to the liter mark.

2. Record the time of day that the cone was filled. T = ________.

3. Allow the waste sample to settle for 45 minutes.

4. Gently spin the cone to facilitate settling of material adhering to the side of the cone.

5. After one hour, record the number of milliliters of settleable solids in the Imhoff cone. Make allowance for voids among the settled material.

6. Record the settleable solids as ml/l or milliliters per liter.

Settleable Solids, Influent = ______ ml/l

Settleable Solids, Effluent = ______ ml/l

Settleable Solids, Removal = ______ ml/l

E. Example

Samples were collected from the influent and effluent of a primary clarifier. After one hour, the following results were recorded:

	Settleable Solids, ml/l
Influent	12.0
Effluent	0.2

F. Calculations

1. Calculate the efficiency or percent removal of the above primary clarifier in removing settleable solids.

$$\text{\% Removal of Set Sol} = \frac{(\text{Infl. Set Sol, ml/l} - \text{Effl. Set Sol, ml/l})}{\text{Influent Set Sol, ml/l}} \times 100\%$$

$$= \frac{12 \text{ ml/l} - 0.2 \text{ ml/l}}{12 \text{ ml/l}} \times 100\%$$

$$\begin{array}{r} 12.0 \\ \underline{-0.2} \\ 11.8 \end{array}$$

$$= \frac{11.8}{12} \times 100\%$$

$$\begin{array}{r} .983 \\ 12\overline{)\,11.8} \\ \underline{10\ 8} \\ 1\ 00 \\ \underline{96} \\ 40 \end{array}$$

$$= 98\%$$

2. Estimate the gallons per day of sludge pumped to a digester from the above primary clarifier if the flow is 1 MGD (1 million gallons per day). In your plant, the Imhoff cone may not measure or indicate the exact performance of your clarifier

or sedimentation tank, but with some experience you should be able to relate or compare your lab tests with actual performance.

Sludge Removed by Clarifier, ml/l

= Influent Set Sol, ml/l - Effluent Set Sol, ml/l

= 12 ml/l - 0.2 ml/l

= 11.8 ml/l

To estimate the gpd (gallons per day) of sludge pumped to a digester, use the following formula:

Sludge to Digester, gpd

= Total Set Sol Removed, ml/l x 1000 x Flow, MGD

$$= 11.8\ \frac{\text{ml}}{\text{M mg}} \times \frac{1000\ \text{mg}}{\text{ml}} \times \frac{1\ \text{M gal}}{\text{day}}$$

= 11,800 gpd

This value may be reduced by 30 to 75% due to compaction of the sludge in the clarifier.

If you figure sludge removed as a percentage (1.18%), the sludge pumped to the digester would be calculated as follows:

$$\frac{1.18\%}{100\%} = \frac{\text{Sludge to Digester, gpd}}{\text{Flow of 1,000,000 gpd}}$$

$$\text{Sludge to Digester, gpd} = \frac{1.18\% \times 1{,}000{,}000\ \text{gpd}}{100\%}$$

$$= 11{,}800\ \text{gpd}$$

G. Clinical Centrifuge

Settleable solids also may be measured by a small clinical centrifuge. A mixed sample is placed in 15 ml graduate API tubes and spun for 15 minutes. The solid deposition in the tip of the tube is related to plant performance for plant control. A centrifuge also is used in Section 16, Suspended Solids, II, Centrifuge.

QUESTION

12.A Estimate the volume of solids pumped to a digester in gallons per day (gpd) if the flow is 1 MGD, the influent settleable solids is 10 ml/l, and the effluent settleable solids is 0.4 ml/l for a primary clarifier.

13. Sludge Age

A. Discussion

Sludge age is a control guide that is widely used and is a rough indicator of the length of time a pound of solids is maintained under aeration in the system. The basis for calculating the sludge age is weight of suspended solids in the mixed liquor in the aeration tank divided by weight of suspended solids added per day to the aerator.

$$\text{Sludge Age, days} = \frac{\text{Suspended Solids in Mixed Liquor, mg/l} \times \text{Aerator Volume in MG} \times 8.34 \text{ lbs/gal}}{\text{SS in Primary Effluent, mg/l}^{*} \times \text{Daily Flow, MGD} \times 8.34 \text{ lbs/gal}}$$

Any significant additional loading placed on the aerator by the digester supernatant liquor must be added to the above loadings by considering the additional flow (MGD) and concentration (mg/l). The selection of the method of determining sludge age is discussed in Chapter 7, Activated Sludge.

B. What is Tested?

Sample	Common Range, mg/l
Suspended solids in aerator and BOD or suspended solids in primary effluent	Depends on process
Sludge age	Conventional process, 2.5 - 6 days

---

* NOTE: Sludge age is calculated by three different methods:

1. Suspended solids in primary effluent, mg/l
2. Suspended solids removed from primary effluent, mg/l, or primary effluent, suspended solids, mg/l - final effluent, suspended solids, mg/l
3. BOD or COD in primary effluent, mg/l

C. Apparatus

See 16, Suspended Solids Test.

D. Reagents

None.

E. Procedure

See 16, Suspended Solids Test.

F. Example

Suspended Solids in Mixed Liquor = 1500 mg/l

Aeration Tank Volume = 0.50 MG

Suspended Solids in Primary Effl. = 100 mg/l

Daily Flow = 2.0 MGD

G. Calculations

$$\text{Sludge Age, days} = \frac{\text{Susp. Solids in Mixed Liquor, mg/l} \times \text{Aerator Vol., MG} \times 8.34\ \text{lbs/gal}}{\text{Susp. Solids in Primary Effl., mg/l} \times \text{Flow, MGD} \times 8.34\ \text{lbs/gal}}$$

$$= \frac{\text{Mixed Liquor Susp. Solids, lbs}}{\text{Primary Effluent SS, lbs/day}}$$

$$= \frac{1500\ \text{mg/l} \times 0.50\ \text{MG} \times 8.34\ \text{lbs/gal}}{100\ \text{mg/l} \times 2.0\ \text{MGD} \times 8.34\ \text{lbs/gal}}$$

$$= \frac{1500 \times 0.50}{100 \times 2.0}$$

$$= \frac{7.5}{2.0}$$

$$= 3.75\ \text{days}$$

QUESTION

13.5 Determine the sludge age in an activated sludge process if the volume of the aeration tank is 200,000 gallons and the suspended solids in the mixed liquor equals 2000 mg/l. The primary effluent SS is 115 mg/l, and the average daily flow is 1.8 MGD.

14. Sludge (Digested) Dewatering Characteristics

A. Discussion

The dewatering characteristics of digested sludge are very important. The better the dewatering characteristics or drainability of the sludge, the quicker it will dry and the less area will be required for sludge drying beds.

B. What is Tested?

	PREFERRED RANGE	
Sample	Method A	Method B
Digested Sludge	Depends on appearance	100-200 ml

C. Apparatus

METHOD A

1000 ml graduated cylinder.

METHOD B

1. Imhoff cone with tip removed.
2. Sand from drying bed.
3. 500 ml beaker.

D. Reagents

None.

E. Procedure

Two methods are presented in this section. Method A relies on a visual observation and is quick and simple. The only problem is that operators on different shifts might record the same sludge draining characteristics differently. Method B requires 24 hours, but the results are recorded by measuring the volume of liquid that passed through the sand. Method B would be indicative of what would happen if you had sand drying beds.

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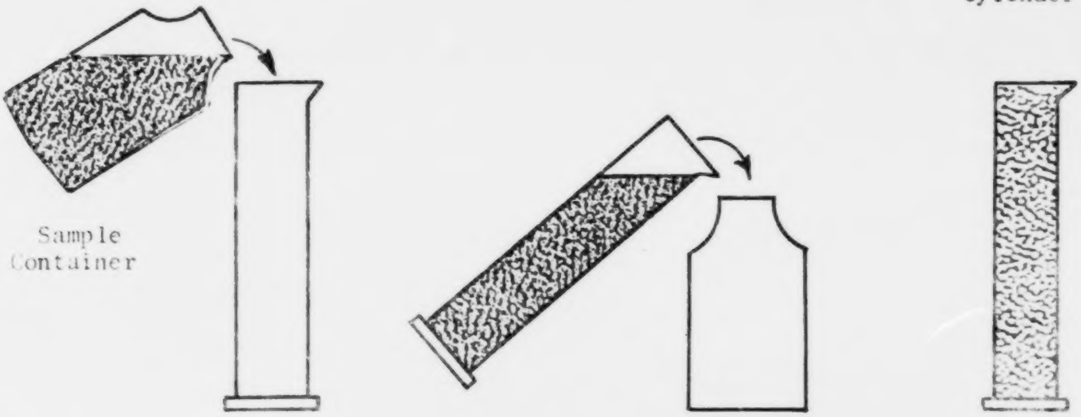
METHOD A

1. Add digested sludge to 1000 ml graduate.
2. Pour sample from graduate back into container.
3. Watch solids adhere to cylinder walls.



1. Add sample of digested sludge to 1000 ml graduate.

2. Pour sample back into sample container. Set graduated cylinder down.

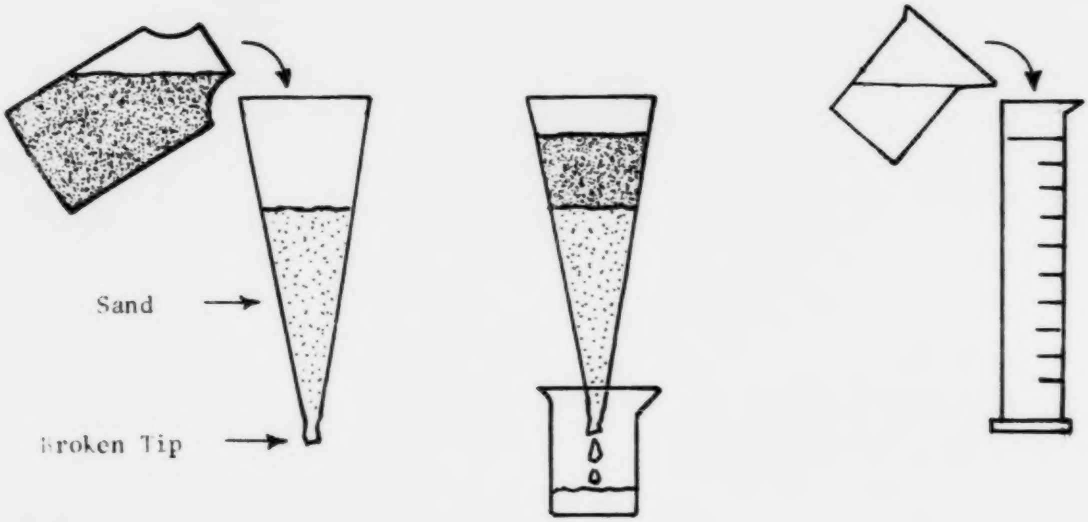
3. Watch graduate. If solids adhere to cylinder wall and water leaves solids in form of rivulets, this is a good dewatering sludge on a sand drying bed (Fig. 14.6).

Fig. 14.6

Sludge on graduated cylinder walls for sludge dewatering test

METHOD B

1. Pour digested sludge on top of sand in Imhoff cone.
2. Place beaker under tip and wait 24 hours.
3. Measure liquid that has passed through the sand.



1. Broken glass Imhoff cone that has tip removed and a glass wool plug in the end to hold the sand in the cone.

2. Fill halfway with sand from sand drying bed.

3. Fill remainder to 1 liter with digested sludge.

4. Place 500 ml beaker under cone tip and wait 24 hours.

5. Record liquid that has passed through sand in ml. If less than 100 ml has passed through sand, you have poor sludge drainability.

QUESTION

14.A What are the differences in the use of (1) a graduated cylinder and (2) an Imhoff cone, filled with sand, that has a broken tip, to measure the dewatering characteristics of digested sludge?

15. Supernatant Graduate Evaluation

A. Discussion

The digester supernatant solids test measures the percent of settleable solids being returned to the plant headworks. The settleable solids falling to the bottom of a graduate should not exceed the bottom 5% of the graduate in most secondary plants. When this happens, you are imposing a load on the primary settling tanks that they were not designed to handle. If the solids exceed 5% you should run a suspended solids Gooch crucible test (Section 16) on the sample and calculate the recycle load on the plant that is originating from the digester.

B. What is Tested?

Sample	Common Values
Supernatant	% Solids should be <5%

C. Apparatus

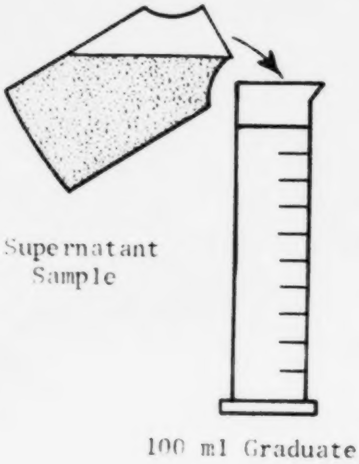
100 ml graduated cylinder.

D. Reagents

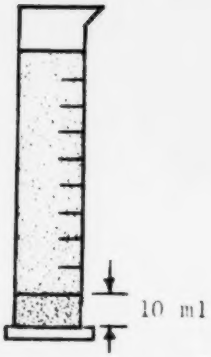
None.

E. Procedure

1. Fill 100 ml graduate with supernatant.



2. After 60 minutes, read ml of solids at bottom.



1. Fill a 100 ml graduated cylinder with supernatant sample.
2. After 60 minutes, read the ml of solids that have settled to the bottom.
3. Calculate supernatant solids, %.

   Supernatant Solids, % = ml of Solids

F. Example

Solids on bottom of cylinder, 10 ml.

G. Calculations

Supernatant Solids, % = ml of Solids

= 10 ml

= 10% Solids (High) by Volume

QUESTION

15.A Why should the results of the supernatant solids test be less than 5% solids?

END OF LESSON 6 OF 8 LESSONS

on

Laboratory Procedures and Chemistry

Work the next portion of discussion and review questions before continuing with Lesson 7.

DISCUSSION AND REVIEW QUESTIONS

(Lesson 6 of 8 Lessons)

Chapter 14. Laboratory Procedures and Chemistry

Name ______________________________ Date ______________

Write the answers to these questions in your notebook. The problem numbering continues from Lesson 5.

22. Calculate the efficiency or percent removal of a primary clarifier when the influent settleable solids are 10 ml/l and the effluent settleable solids are 0.3 ml/l.

23. Why does the actual volume of sludge pumped from a clarifier not agree exactly with calculations based on the settleable solids test?

24. What does sludge age measure?

25. Why should the dewatering characteristics of digested sludge be measured?

26. What happens to the plant when the supernatant from the digester is high in solids?

CHAPTER 14. LABORATORY PROCEDURES AND CHEMISTRY

(Lesson 7 of 8 Lessons)

16. Suspended Solids

I. GOOCH CRUCIBLE

A. Discussion

One of the tests run on wastewater is to determine the amount of material suspended within the sample. The result obtained from the suspended solids test does not mean that all of the suspended solids settle out in the primary clarifier or, for that matter, in the final clarifier. Some of the particles are of such size and weight that they will not settle without additional treatment. Therefore, suspended solids are a combination of settleable solids and those solids that remain in suspension.

B. What is Tested?

Sample	Common Ranges, mg/l
Influent	Weak 150 - 400+ Strong
Primary Effluent	Weak 60 - 150+ Strong
Secondary Effluent	10 Good - 60+ Bad
Activated Sludge Tests	Depending on Type of Process
Mixed Liquor	1000 - < 5,000
Return or Waste Sludge	2000 - < 12,000
Digester Tests:	
Supernatant	3000 - < 10,000

When supernatant suspended solids are greater than 10,000 mg/l, the total solids test is usually performed.

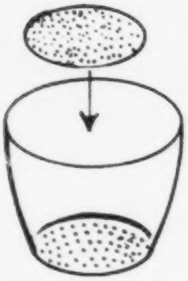
C. Apparatus

1. 2.4 cm glass fiber filter.
2. No. 4 Gooch crucible.
3. Distilled water.
4. Filter flask.
5. Graduated cylinder.
6. Vacuum pump or aspirator.
7. Oven.
8. Analytical balance.

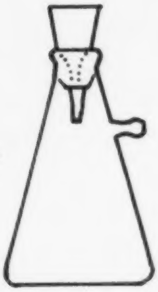
D. Outline of Procedure

The procedure is outlined on Page 14-157.

(Method with Gooch Crucible and Glass Fiber Filter)

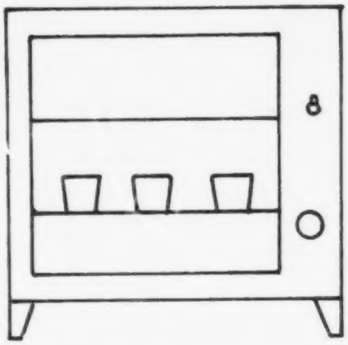


1. Insert glass fiber filter.

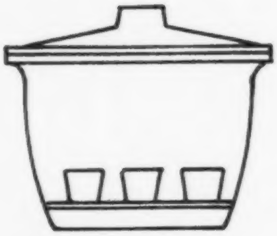


Filtering Flask

2. Seat filter, by adding distilled water and applying vacuum.

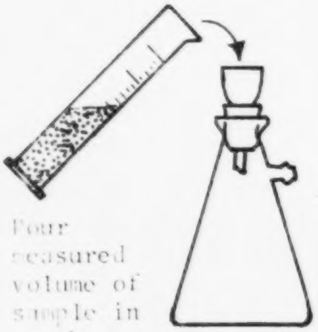


3. Dry crucibles in oven at 103°C.



4. Cool.

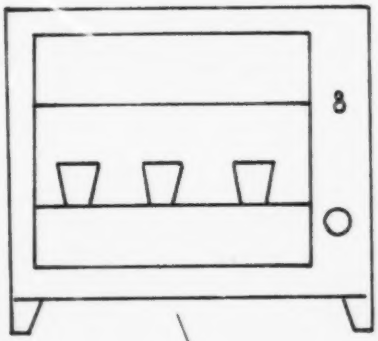
5. Weigh crucible.



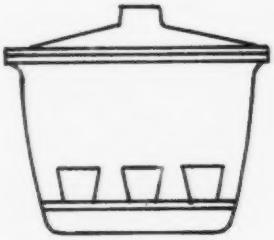
6. Pour measured volume of sample in Gooch crucible.

7. Filter out suspended solids with vacuum.

8. Wash graduate, crucible, and filter with distilled water to complete solids transfer.



9. Dry crucibles plus suspended solids at 103°C.



10. Cool.

11. Weigh crucible plus suspended solids.

E. Preparation of Gooch Crucible

1. Put a No. 4 Gooch crucible into filtering apparatus.

2. Insert 2.4 cm glass fiber filter and center it.

3. Apply suction.

4. Wash filter with 100 ml of distilled water to seat well.

5. Dry at 103°C for one hour.

6. If volatile suspended solids are to be determined, ignite crucible in muffle furnace for one hour at 550°C.

7. Cool in desiccator.

8. Weigh and record tare weight.

F. How to Perform the Test

1. Depending on the suspended solids content, measure out a 25, 50, or 100 ml portion of a well mixed sample into a graduated cylinder. Use 25 ml if sample filters slowly. Use larger volumes of sample if samples filter easily, such as secondary effluent. Try to limit filtration time to about 15 minutes or less.

2. Wet prepared Gooch crucible with distilled water and apply suction.

3. Filter sample through the Gooch crucible.

4. Wash out dissolved solids on the filter with about 20 ml of distilled water. (Use two 10 ml portions.)

5. Dry crucible at 103°C for one hour or other specified time. Some samples may require up to three hours to dry if the residue is thick.

6. Cool crucible in desiccator for 20-30 minutes.

7. Weigh and record weight.

8. Total Weight = ________ g

   Tare Weight = ________ g

   Solids Weight = ________ g

G. Precautions

1. Check and regulate the oven temperature at 103° - 105°C.

2. Observe crucible and glass fiber for any possible leaks. A leak will cause solids to pass through and give low results. The glass fiber filter may become unseated and leaky when the crucible is placed on the filter flask. The filter should be reseated by adding distilled water to the filter in the crucible and applying vacuum before filtering the sample.

3. Mix the sample thoroughly so that it is completely uniform in suspended solids when measured into a graduated cylinder before sample can settle out. This is especially true of samples heavy in suspended solids, such as raw wastewater and mixed liquor in activated sludge which settle rapidly. The test can be no better than the mix.

4. It is a good practice to prepare a number of extra Gooch crucibles for additional tests if the need arises. If a test result appears faulty or questionable, the test should be repeated. Check filtration rate and clarity of water passing through the filter.

H. Example and Calculations

This section is provided to show you the detailed calculations. After some practice, most operators use the lab work sheet as shown at the end of the calculations.

CALCULATIONS FOR SUSPENDED SOLIDS TEST

(or use lab work sheet at end of calculations)

Example: Assume the following data.

Volume of sample = 50 ml.

	Recorded Weights
Crucible weight	21.6329 g
Crucible plus dry solids	21.6531 g
Crucible plus ash[11]	21.6360 g

---

[11] Obtained by placing the crucible plus dry solids in a muffle furnace at 550°C for one hour. The crucible plus remaining ash are cooled and weighed.

1. Compute total suspended solids.

21.6531 g	Weight of Crucible plus Dry Solids, grams
- 21.6329 g	- Weight of Crucible, grams
= 0.0202 g	= Weight of Dry Solids, grams

or

= 20.2 mg

1000 milligrams (mg) = 1 gram (g)

or

20.2 mg = 0.0202 g

$$\text{Total Suspended Solids, mg/l} = \frac{\text{Weight of Dry Solids, mg} \times 1000 \text{ ml/l}}{\text{Sample Volume, ml}}$$

$$= 20.2 \text{ mg} \times \frac{1000 \text{ ml/l}}{50 \text{ ml}}$$

$$= 404 \text{ mg/l}$$

404.
50 / 20200.
200
200
200

2. Compute volatile or organic suspended solids.

21.6531 g	Weight of Crucible plus Dry Solids, g
- 21.6360 g	- Weight of Crucible plus Ash, g
= 0.0171 g	= Weight of Volatile Solids, g

or

= 17.1 mg

$$\text{Volatile Suspended Solids, mg/l} = \frac{\text{Weight of Volatile Solids, mg} \times 1000 \text{ ml/l}}{\text{Sample Volume, ml}}$$

$$= \frac{17.1 \text{ mg} \times 1000 \text{ ml/l}}{50 \text{ ml}}$$

$$= 342 \text{ mg/l}$$

342
50 / 17100
150
210
200
100
100

3. Compute the percent volatile solids.

$$\text{Volatile Solids, \%} = \frac{(\text{Weight Volatile, mg})\ 100\%}{\text{Weight Total Dry Solids, mg}}$$

$$= \frac{17.1\ \text{mg}}{20.2\ \text{mg}} \times 100\%$$

$$= 84.7\%$$

$$\begin{array}{r|l} & .8465 \\ \hline 20.2 & 17.10 \\ & \underline{16\ 16} \\ & 940 \\ & \underline{808} \\ & 1320 \\ & \underline{1212} \\ & 1080 \\ & 1010 \end{array}$$

4. Compute fixed or inorganic suspended solids.

21.6360 g	Weight of Crucible plus Ash, g
- 21.6329 g	- Weight of Crucible, g
= 0.0031 g	= Weight of Fixed Solids, g

or

= 3.1 mg

$$\text{Fixed Suspended Solids, mg/l} = \frac{\text{Weight of Fixed Solids, mg} \times 1000\ \text{ml/l}}{\text{Sample Volume, ml}}$$

$$= \frac{3.1\ \text{mg} \times 1000\ \text{ml/l}}{50\ \text{ml}}$$

$$= 62\ \text{mg/l}$$

To check your work:

Fixed Susp. Solids = Total Susp. Solids, mg/l - Volatile Susp. Solids, mg/l

= 404 mg/l - 342 mg/l

= 62 mg/l (Check)

$$\begin{array}{r} 404 \\ \underline{-342} \\ 62 \end{array}$$

5. Compute the percent fixed solids.

$$\text{Fixed Solids, \%} = \frac{(\text{Weight Fixed, mg}) \times 100\%}{\text{Weight Total, mg}}$$

$$= \frac{3.1\ \text{mg}}{20.2\ \text{mg}} \times 100\%$$

$$= 15.3\%$$

The above calculations are also performed on a Laboratory Work Sheet (Fig. 14.7) to illustrate the use of the work sheet.

CALCULATIONS FOR OVERALL PLANT REMOVAL OF SUSPENDED SOLIDS IN PERCENT

Example: Assume the following data.

Influent suspended solids	202 mg/l
Primary Effluent suspended solids	110 mg/l
Secondary Effluent suspended solids	52 mg/l
Final Effluent suspended solids	12 mg/l

To calculate the percent removal or treatment efficiency for a particular process or the overall plant, use the following formula:

$$\text{Removal, \%} = \frac{(\text{In} - \text{Out})}{\text{In}} \times 100\%$$

Compute percentage removed between influent and primary effluent:

$$\text{Removal, \%} = \frac{(\text{In} - \text{Out})}{\text{In}} \times 100\%$$

$$= \frac{(202\ \text{mg/l} - 110\ \text{mg/l})}{202\ \text{mg/l}} \times 100\%$$

$$= \frac{92}{202} \times 100\% \qquad \begin{array}{r} 202 \\ -110 \\ \hline 92 \end{array}$$

$$= 45.5\%$$

Compute percentage removed between influent and secondary effluent:

$$\text{Removal, \%} = \frac{(\text{In} - \text{Out})}{\text{In}} \times 100\%$$

$$= \frac{(202 \text{ mg/l} - 52 \text{ mg/l})}{202 \text{ mg/l}} \times 100\% \qquad \begin{array}{r} 202 \\ \underline{-52} \\ 150 \end{array}$$

$$= \frac{150}{202} \times 100\% \qquad \begin{array}{r} .74 \\ 202\overline{)\,150.00} \\ \underline{141.4} \\ 8\;60 \\ \underline{8\;08} \\ 52 \end{array}$$

$$= 74\%$$

Compute percentage removed between influent and final effluent (overall plant percentage removed):

$$\text{Removal, \%} = \frac{(\text{In} - \text{Out})}{\text{In}} \times 100\%$$

$$= \frac{(202 \text{ mg/l} - 12 \text{ mg/l})}{202 \text{ mg/l}} \times 100\%$$

$$= \frac{190}{202} \times 100\%$$

= 94.1% removal for the plant in suspended solids

CALCULATIONS FOR POUNDS SUSPENDED SOLIDS REMOVED PER DAY

Example: Assume the following data.

Influent suspended solids	200 mg/l
Effluent suspended solids	10 mg/l
Flow in million gallons/day	2 MGD
1 gallon of water weighs	8.34 lbs

Compute pounds suspended solids removed:

The general formula for computing pounds removed is

Material Removed, lbs/day = (Concentration In, mg/l - Concentration Out, mg/l) x Flow, MGD x 8.34 lb/gal

= (200 mg/l - 10 mg/l) x 2 MGD x 8.34 lb/gal

= 190 x 2 x 8.34

= 3169 lbs/day of suspended solids removed by plant

```
   8.34
    380
  -----
    000
   6672
  2502
  -------
3169.20
```

DERIVATION

This section is not essential to efficient plant operation, but is provided to furnish you with a better understanding of the calculation if you are interested. For practical purposes,

1 mg/l = 1 ppm or 1 part per million

or = 1 mg/million mg, because 1 liter = 1,000,000 mg

Therefore:

$$\frac{\text{lbs}}{\text{day}} = \frac{\text{mg}}{\text{M mg}} \times \frac{\text{M gal}}{\text{day}} \times \frac{\text{lbs}}{\text{gal}}$$

$$= \text{lbs/day}$$

(Suspended Solids - Gooch)

PLANT CLEAN WATER

DATE ____________

SUSPENDED SOLIDS & DISSOLVED SOLIDS

SAMPLE	INFL.							
Crucible	#015							
Ml Sample	50							
Wt Dry & Dish Wt Dish	21.6531 21.6329							
Wt Dry	0.0202							
mg/l = $\frac{\text{Wt Dry, gm} \times 1{,}000{,}000}{\text{Ml Sample}}$	404 mg/l							
Wt Dish & Dry Wt Dish & Ash	21.6531 21.6360							
Wt Volatile	0.0171							
% Vol = $\frac{\text{Wt Vol}}{\text{Wt Dry}} \times 100\%$	84.7%							

BOD

Blank ____________

SAMPLE										
DO Sample										
Bottle #										
% Sample										
Blank or adj blank DO after incubation										
Depletion, 5 days										
Dep %										

Nitrate NO_3

Sample ____________ ____________

Graph Reading ____________ ____________

Sett. Solids

Sample ____________ ____________

Direct Ml/l ____________ ____________

COD

Sample ____________ ____________ ____________

Blank Titration ____________ ____________ ____________

Sample Titration ____________ ____________ ____________

Depletion ____________ ____________ ____________

mg/l = $\frac{\text{Dep} \times N \text{ FAS} \times 8000}{\text{Ml Sample}}$ ____________ ____________ ____________

Fig. 14.7 Calculation of solids content on Laboratory Work Sheet

(Suspended Solids - Gooch)

TOTAL SOLIDS

SAMPLE						
Dish No.						
Wt Dish & Wet Wt Dish						
Wt Wet						
Wt Dish + Dry Wt Dish						
Wt Dry						
% Solids = $\frac{\text{Wt Dry x 100\%}}{\text{Wt Wet}}$						
Wt Dish + Dry Wt Dish + Ash						
Wt Volatile						
% Volatile = $\frac{\text{Wt Vol x 100\%}}{\text{Wt Dry}}$						
pH						
Vol. Acid						
alinity as $CaCO_3$						

Grease (Soxlet) ____________

Sample ____________

Ml Sample ____________

Wt Flask + Grease

Wt Flask ____________

Wt Grease ____________

mg/l = $\frac{\text{Wt Grease, mg x 1000}}{\text{Ml Sample}}$ ____________

H_2S (Gas) (Starch-Iodine)

Blank ________ Ml

Sample ________ Ml

Diff ________ Ml

Diff x .68 ________ mg/l

mg/l x 43.6 ________ grain/100 cu ft

Fig. 14.7 Calculation of solids content on Laboratory Work Sheet (continued)

QUESTIONS

16.A Why does some of the suspended material in wastewater fail to be removed by settling or flotation within one hour?

16.B Given the following data:

100 ml of sample

Crucible weight	19.3241 g
Crucible plus dry solids	19.3902 g
Crucible plus ash	19.3469 g

Compute:

a. Total suspended solids
b. Volatile suspended solids
c. Percent volatile
d. Fixed suspended solids
e. Percent fixed

16.C Compute the percent removal of suspended solids by the primary clarifier, secondary process (removal between primary effluent and secondary effluent), and overall plant:

Influent suspended solids = 221 mg/l
Primary effluent SS = 159 mg/l
Final effluent SS = 33 mg/l

16.D If the data in problem 16.C is from a 1.5 MGD plant, calculate the pounds of suspended solids removed:

a. By the primary unit
b. By the secondary unit
c. By the overall plant

II. CENTRIFUGE

A. Discussion

This procedure is frequently used in plants as a quick and easy method to estimate the suspended solids concentration of the mixed liquor in the aeration tank instead of the regular suspended solids test. Many operators control the solids in their aerator on the basis of centrifuge readings. Others prefer to control solids using Fig. 14.8. In either case, the operator should periodically compare centrifuge readings with values obtained from suspended solids tests. If the solids are in a good settling condition, a 1% centrifuge solids reading could have a suspended solids concentration of 1000 mg/l. However, if the sludge is feathery, a 1% centrifuge solids reading could have a suspended solids concentration of 600 mg/l.

The centrifuge reading versus mg/l suspended solids chart (Fig 14.8) must be developed for each plant by comparing centrifuge readings with suspended solids determined by the regular Gooch crucible method. The points are plotted and a line of best fit is drawn as shown in Fig. 14.8. This line must be periodically checked by comparing centrifuge readings with regular suspended solids tests because of the large number of variables influencing the relationship, such as characteristics of influent waste, mixing in aerator, and organisms in aerator. If you don't have a centrifuge or if your solids content is over 1500 mg/l, determine suspended solids by the regular method.

B. What is Tested?

Sample	Common Range
Suspended Solids in Mechanical Aeration Tanks	800 - 1200 mg/l
Suspended Solids in Diffused Aeration Tanks	1000 - 3000 mg/l

C. Apparatus

1. Centrifuge.

2. Graduated centrifuge tubes, 15 ml.

D. Reagents

None.

E. Procedure

1. Collect sample in regular sampling can.
2. Mix sample well and fill each centrifuge tube to the 15 ml line with sample.
3. Place filled sample tubes in centrifuge holders.
4. Crank centrifuge at fast speed as you count slowly to 60. Be sure to count and crank at the same speed for all tests. It is extremely important to perform each step exactly the same every time.
5. Remove one tube and read the amount of suspended solids concentrated in the bottom of the tube. This reading will be 1/10 of ml. Results in other tubes should be compared.
6. Refer to the conversion graph to determine suspended solids in mg/l.

NOTE: The reason for filling tubes to the 15 ml mark is that the graph (Fig. 14.8) is computed for samples of this size.

F. Example

Suspended solids concentration on bottom of centrifuge tube is 0.4 ml.

G. Calculations

From Fig. 14.8, find 0.4 ml on centrifuge reading side and follow line horizontally to line on chart. Drop downward from line on chart to mg/l suspended solids and read result of 900 mg/l.

If the suspended solids concentration is above or below the desired range, then you should make the proper changes in the pumping rate of the waste and return sludge. For details on controlling the solids concentration, refer to Chapter 7, Activated Sludge.

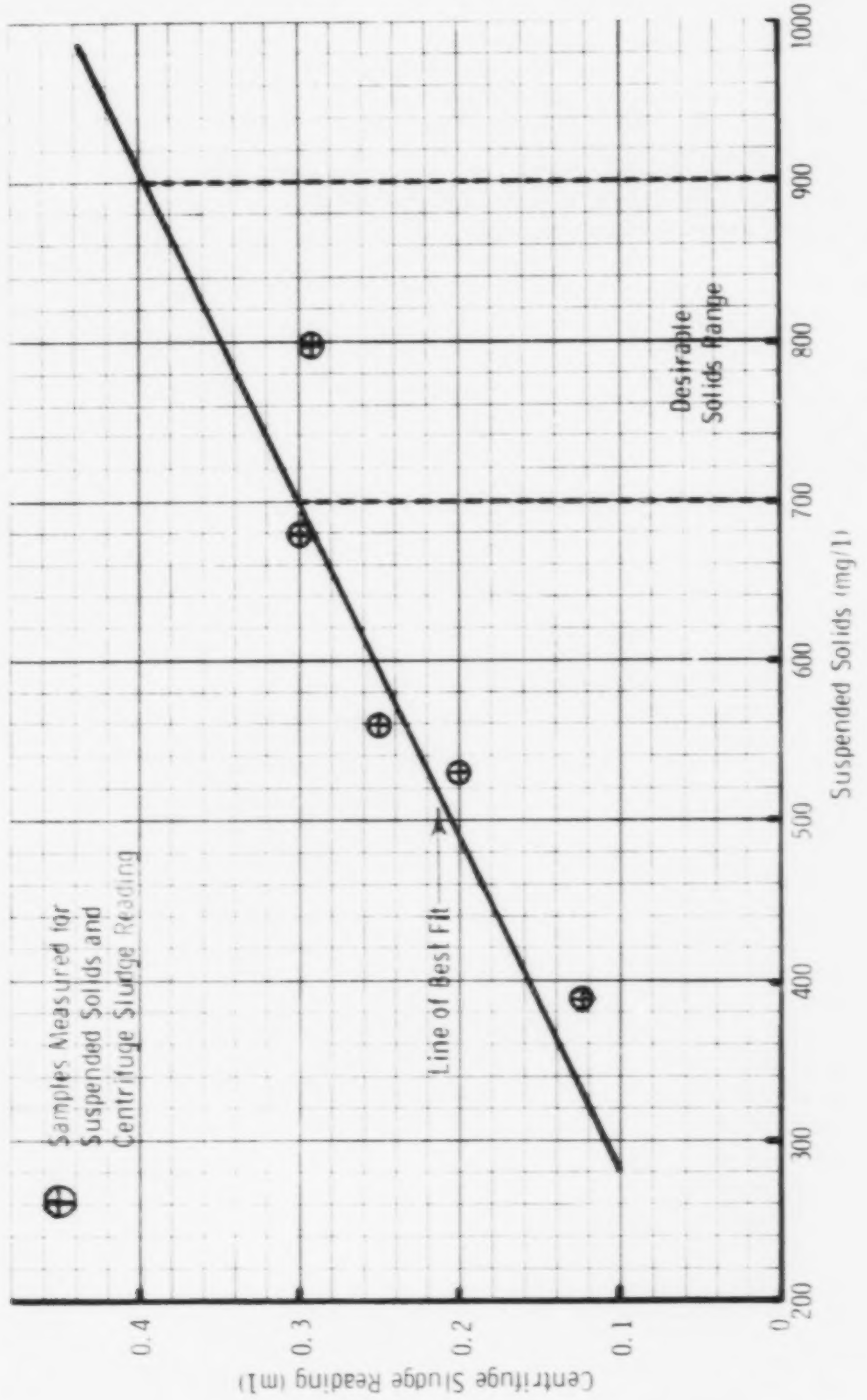


Fig. 14.8 Plant control by centrifuge solids in aeration tank, centrifuge speed, 1750 RPM.

H. Development of Fig. 14.8

To develop Fig. 14.8 take a sample from the aeration tank and measure suspended solids and also centrifuge a portion of the sample to obtain the centrifuge sludge reading in ml of sludge at the bottom of the tube. Obtain other samples of different solids concentrations to obtain the points on the graph. Draw a line of best fit through the points. Periodically the points should be checked because the influent characteristics and conditions in the aeration tank change.

QUESTION

16.E What is the advantage of the centrifuge test for determining suspended solids in an aeration tank in comparison with other methods of measuring suspended solids?

17. Temperature

I. WASTEWATER

A. Discussion

This is one of the most frequently taken tests. One of the many uses is to calculate the percent saturation of dissolved oxygen in the DO test. (Refer to DO Test for procedure.)

Changes of plus or minus 4°F from the average or expected value should be investigated and the cause corrected if possible.

For example, an influent temperature drop may indicate large volumes of cold water from infiltration. An increase in temperature may indicate hot water discharged by industry is reaching your plant.

A temperature measurement should be taken where samples are collected for other tests. This test is always immediately performed on a grab sample because it changes so rapidly. Always leave the thermometer in the liquid while reading the temperature. Record temperature on suitable work sheet, including time, location, and sampler's name.

B. What is Tested?

Sample	Common Range
Influent[12]	65°F to 85°F[13]
Effluent[12]	60°F to 95°F or higher from ponds
Receiving Water[12]	60°F to ambient temperature[14]
Digester (Recirculated Sludge before Heat Exchanger--Supernatant)	60°F to 100°F

---

[12] If dissolved oxygen (DO) measurements are performed on any samples, the temperature should be measured and recorded.

[13] Depends on season, location, and temperature of water supply.

[14] Ambient Temperature (AM-bee-ent). Temperature of the surroundings.

C. Apparatus

1. One NBS (National Bureau of Standards) thermometer for calibration of the other thermometers.
2. One Fahrenheit mercury-filled, 1° subdivided thermometer.
3. One Celsius (formerly called Centigrade) mercury-filled, 1° subdivided thermometer.
4. One metal case to fit each thermometer.

There are three types of thermometers and two scales.

Scales

1. Fahrenheit, marked °F.
2. Celsius, marked °C (formerly Centigrade).

Thermometers

1. Total immersion. This type of thermometer must be totally immersed when read. This will change most rapidly when removed from the liquid to be recorded.
2. Partial immersion. This type thermometer will have a solid line around the stem below the point where the scale starts.
3. Dial. This type has a dial that can be easily read while the thermometer is still immersed. Dial thermometer readings should be checked (calibrated) against the NBS thermometer. Some dial thermometers can be recalibrated (adjusted) to read the correct temperature of the NBS thermometer.

D. Reagents

None.

E. Procedures

Use a large volume of sample, preferably at least a 2-pound coffee can or equivalent volume. The temperature will have less chance to change in a large volume than in a small container. Collect sample in container and immediately measure and record temperature. Do not touch the bottom or sides of the sample container with the thermometer. To avoid breaking or damaging glass thermometer, store it in a shielded metal case. Check your thermometer accuracy against the NBS certified thermometer by measuring the temperature of a sample with both thermometers simultaneously. Some of the poorer quality thermometers are substantially inaccurate (off as much as 6°F).

F. Example

To measure influent temperature, obtain sample in large coffee can, immediately immerse thermometer in can, and record temperature when reading becomes constant. For example, 72°F.

G. Calculations

Normally, we measure and record temperatures using a thermometer with the proper scale. However, we could measure a temperature in °F and convert to °C, or we might measure a temperature in °C and convert to °F. The following formulas are used to convert temperatures from one scale to the other.

1. Measure in °F, want °C:

$$^{\circ}C = 5/9\ (^{\circ}F - 32^{\circ})$$

2. Measure in °C, want °F:

$$^{\circ}F = 9/5\ (^{\circ}C) + 32^{\circ}$$

3. Example Calculation:

The measured influent temperature was 77°F.
What was the temperature in °C?

$$
\begin{aligned}
{}^{\circ}C &= 5/9\,({}^{\circ}F - 32^{\circ}) \\
&= 5/9\,(77^{\circ} - 32^{\circ}) \\
&= 5/9\,(45^{\circ}) \\
&= 25^{\circ}
\end{aligned}
$$

$$\begin{array}{r} 77 \\ -32 \\ \hline 45 \end{array}$$

$$\begin{array}{r} 5 \\ 9\overline{)45} \end{array}$$

$$\begin{array}{r} 5 \\ \times 5 \\ \hline 25 \end{array}$$

QUESTIONS

17.A What could a change in influent temperature indicate?

17.B Why should the thermometer remain immersed in the liquid while being read?

17.C Why should thermometers be calibrated against an accurate NBS certified thermometer?

17. Temperature

II. DIGESTER SLUDGE

A. Discussion

The rate of sludge digestion in a digester is a function of the digester temperature. The normal temperature range in a digester is around 95 to 98°F. The temperature of a digester should not be changed by more than 1°F per day because then the helpful organisms in the digester are unable to adjust to rapid temperature changes.

B. Apparatus and Procedure

Refer to I., WASTEWATER.

END OF LESSON 7 OF 8 LESSONS

on

Laboratory Procedures and Chemistry

Work the next portion of the discussion and review questions before continuing with Lesson 8.

DISCUSSION AND REVIEW QUESTIONS

(Lesson 7 of 8 Lessons)

Chapter 14. Laboratory Procedures and Chemistry

Name ______________________________ Date ____________

Write the answers to these questions in your notebook. The problem numbering continues from Lesson 6.

27. Given the following data:

100 ml of sample	
Crucible weight	19.9850 g
Crucible plus dry solids	20.0503 g
Crucible plus ash	20.0068 g

Compute:

1. Total suspended solids
2. Volatile suspended solids
3. Percent volatile

28. Estimate the pounds of solids removed per day by a primary clarifier if the influent suspended solids is 220 mg/l and the effluent suspended solids is 120 mg/l when the flow is 1.5 MGD.

29. What is the ambient temperature?

30. Convert a temperature reading of 50°F to °C.

31. Why should the temperature of a digester not be changed by more than 1°F per day?

CHAPTER 14. LABORATORY PROCEDURES AND CHEMISTRY

(Lesson 8 of 8 Lessons)

18. Total and Volatile Solids (Sludge)

A. Discussion

Total solids measure the combined amount of suspended and dissolved materials in the sample.

This test is used for wastewater sludges or where the solids can be expressed in percentages by weight and the weight can be measured on an inexpensive beam balance to the nearest .01 of a gram. The total solids are composed of two components, volatile and fixed solids. Volatile solids are composed of organic compounds which are of either plant or animal origin. Fixed solids are inorganic compounds such as sand, gravel, minerals, or salts.

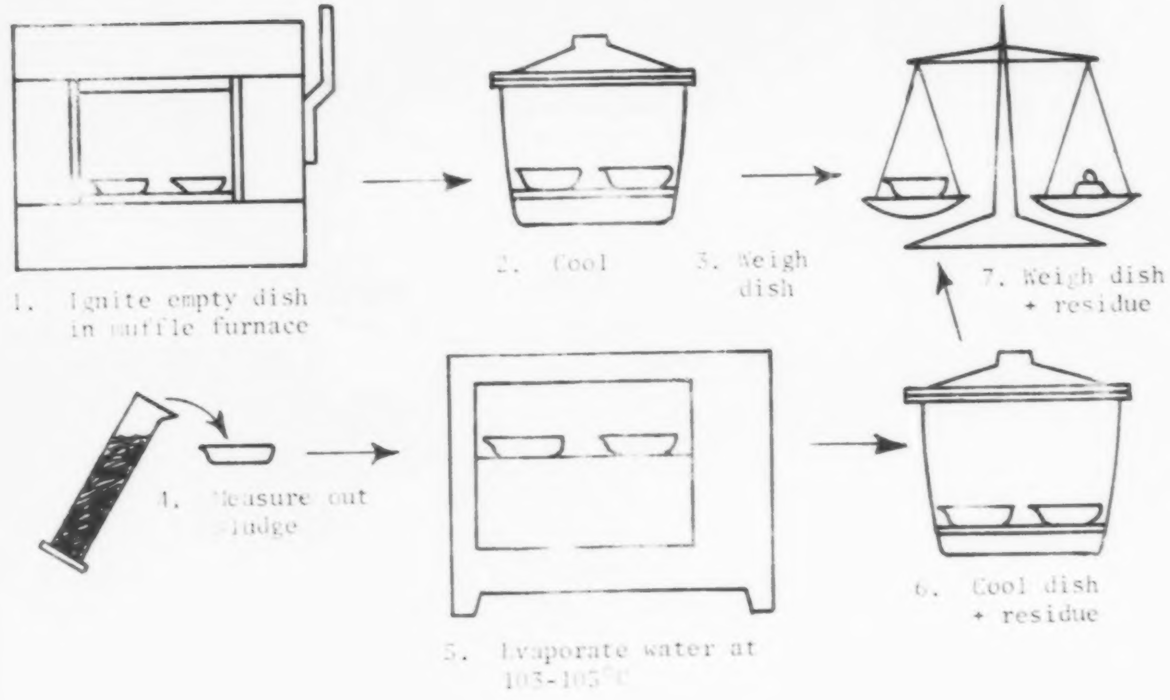
B. What is Tested?

	COMMON RANGE, % BY WEIGHT		
Sample	Total	Volatile	Fixed
Raw Sludge	6% to 9%	75%	25% ± 6%
Raw Sludge plus Waste Activated Sludge	2% to 5%	80%	20% ± 5%
Recirculated Sludge	1.5% to 3%	75%	25% ± 5%
Supernatant:			
Good Quality, has Suspended Solids	< 1%	50%	50% ± 10%
Poor Quality	> 5%		
Digested Sludge to Air Dry	3% too Thin to < 8%	50%	50% ± 10%

C. Apparatus

1. Evaporating dish.
2. Analytical balance.
3. Drying oven, 103° - 105°C.
4. Measuring device--graduated cylinder.
5. Muffle furnace, 550°C.

D. Outline of Procedure


1. Ignite empty dish in muffle furnace
2. Cool
3. Weigh dish
7. Weigh dish + residue
4. Measure out sludge
5. Evaporate water at 103-105°C
6. Cool dish + residue

PROCEDURE

1. Dry the dish by ignition in a muffle furnace at 550°C for one hour. Cool dish in desiccator.

2. Tare the evaporating dish to the nearest 10 milligrams, or 0.01 g on the Mettler single pan balance. Record the weight as Tare Weight = ________________ gms.

3. Weigh dish plus 50 to 100 ml of well mixed sludge sample. Record total weight to nearest 0.01 gram as Gross Weight = __________ gms.

4. Evaporate the sludge sample to dryness in the 103°C drying oven.

5. Weigh the dried residue in the evaporating dish to the nearest 10 milligrams, or 0.01 g. Record the weight as Dry Sample and Dish = __________ gms.

6. Compute the net weight of the residue by subtracting the tare weight of the dish from the dry sample and dish.

E. Precautions

1. Be sure that the sample is thoroughly mixed and is representative of the sludge being pumped. Generally, where sludge pumping is intermittent, sludge is much heavier at the beginning and is less dense toward the end of pumping. Take several equal portions of sludge at regular intervals and mix for a good sample.

2. Take a large enough sample. Measuring a 50 or 100 ml sample which is closely equal to 50 or 100 grams is recommended. Since this material is so heterogeneous (non-uniform), it is difficult to obtain a good representative sample with less volume. Smaller volumes will show greater variations in answers, due to the uneven and lumpy nature of the material.

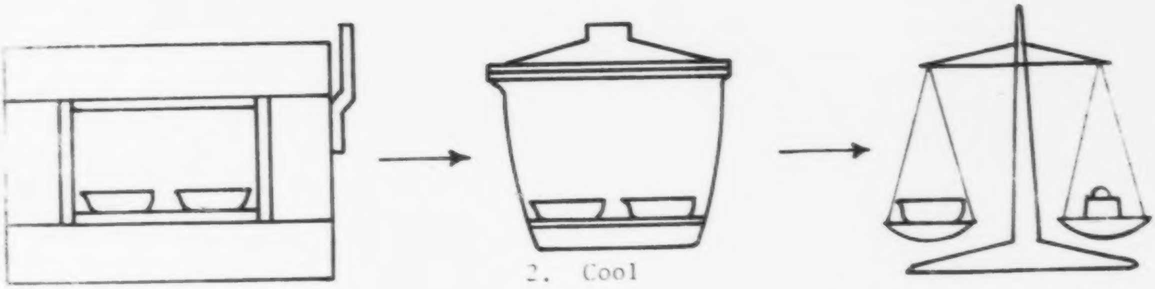
3. Control oven temperature closely at 103° - 105°C. Some solids are lost at any drying temperature. Close control of oven temperature is necessary because higher temperatures increase the losses of volatile solids in addition to the evaporated water.

4. Heat dish long enough to insure evaporation of water, usually about 3-4 hours. If heat drying and weighing are repeated, stop when the weight change becomes small per unit of drying time. The oxidation, dehydration, and degradation of the volatile fraction won't completely stabilize until it is carbonized or becomes ash.

5. Since sludge is so non-uniform, weighing on the analytical balance should probably be made only to the nearest 0.01 grams or 10 milligrams.

F. Outline of Procedure for Volatile Solids

(continue from total solids test)



PROCEDURE

1. Determine the total solids as previously described in Section D.

2. Ignite the dish and residue from total solids test at 550°C for one hour or until a white ash remains.

3. Cool in desiccator for about 30 minutes.

4. Weigh and record weight of Dish Plus Ash = ____________ gms.

G. Example

Weight of Dish (Tare)	=	20.31 g
Weight of Dish plus Wet Solids (Gross)	=	70.31 g
Weight of Dish plus Dry Solids	=	22.81 g
Weight of Dish plus Ash	=	20.93 g

H. Calculations

See Laboratory Work Sheet (Fig. 14.9) or calculations shown below.

1. Find weight of sample.

Weight of Dish plus Wet Solids (Gross)	=	70.31 g
Weight of Dish (Tare)	=	20.31 g
Weight of Sample	=	50.00 g

2. Find weight of total solids.

Weight of Dish plus Dry Solids	=	22.81 g
Weight of Dish (Tare)	=	20.31 g
Weight of Total Solids	=	2.50 g

3. Find % solids.

$$\text{\% Solids} = \frac{(\text{Weight of Solids, g})\ 100\%}{\text{Weight of Sample, g}}$$

$$= \frac{(2.50\ \text{g})\ 100\%}{50.00\ \text{g}}$$

$$= 5\%$$

4. Find weight of volatile solids.

Weight of Dish plus Dry Solids	=	22.81 g
Weight of Dish plus Ash	=	20.93 g
Weight of Volatile Solids	=	1.88 g

5. Find % volatile solids.

$$\% \text{ Volatile Solids} = \frac{(\text{Weight of Volatile Solids, g})\ 100\%}{\text{Weight of Total Solids, g}}$$

$$= \frac{(1.88\ g)\ 100\%}{2.50\ g}$$

$$= 76\%$$

QUESTION

18.A What is the origin of the volatile solids found in a digester?

18.B What is the significance of volatile solids in a treatment plant?

19. Turbidity

See Clarity.

PLANT ______________________

DATE ______________________

SUSPENDED SOLIDS & DISSOLVED SOLIDS

SAMPLE							
Crucible							
Ml Sample							
Wt Dry & Dish Wt Dish							
Wt Dry							
mg/l = $\frac{\text{Wt Dry, gm} \times 1,000,000}{\text{Ml Sample}}$							
Wt Dish & Dry Wt Dish & Ash							
Wt Volatile							
% Vol = $\frac{\text{Wt Vol}}{\text{Wt Dry}}$ x 100%							

BOD

Blank __________

SAMPLE										
Ml Sample										
Bottle #										
% Sample										
Blank or adj blank DO after incubation										
Depletion, 5 days										
Dep %										

Nitrate NO_3

Sample __________ __________

Graph Reading __________ __________

Sett. Solids

Sample __________ __________

Direct Ml/l __________ __________

COD

Sample

Blank Titration __________ __________ __________

Sample Titration __________ __________ __________

Depletion __________ __________ __________

__________ __________ __________

mg/l = $\frac{\text{Dep} \times N\ \text{FAS} \times 8000}{\text{Ml Sample}}$ __________ __________ __________

Fig. 14.9 Calculation of total solids on Laboratory Work Sheet

TOTAL SOLIDS

SAMPLE	RAW					
Dish No.	7					
Wt Dish & Wet Wt Dish	70.31 20.31					
Wt Wet	50.00					
Wt Dish + Dry Wt Dish	22.81 20.31					
Wt Dry	2.50					
% Solids = $\frac{\text{Wt Dry x 100\%}}{\text{Wt Wet}}$	5.0%					
Wt Dish + Dry Wt Dish + Ash	22.81 20.93					
Wt Volatile	1.88					
% Volatile = $\frac{\text{Wt Vol x 100\%}}{\text{Wt Dry}}$	76%					
pH						
Vol. Acid						
Alkalinity as $CaCO_3$						

Grease (Soxlet) ____________________

Sample ____________________

Ml Sample ____________________

Wt Flask + Grease
Wt Flask ____________________

Wt Grease ____________________

mg/l = $\frac{\text{Wt Grease, mg x 1000}}{\text{Ml Sample}}$ ____________________

H_2S (Gas) (Starch-Iodine)

Blank ________ Ml

Sample ________ Ml

Diff ________ Ml

Diff x .68 ________ mg/l

mg/l x 43.6 ________ grain/100 cu ft

Fig. 14.9 Calculation of total solids on Laboratory Work Sheet (continued)

20. Volatile Acids and Total Alkalinity

A. Discussion

Volatile acids are determined on sludge samples from the digesters. Most modern digesters have sampling pipes where you can draw a sample from various levels of the tank. Be sure to allow the sludge in the line to run for a few minutes in order to obtain a representative sample of the digester contents. Samples also may be collected from supernatant draw-off tubes, or thief holes.[15]

The concentrations of volatile acids and alkalinity are the first measurable changes that take place when the process of digestion is becoming upset. The volatile acid/alkalinity relationship can vary from 0.1 to about 0.5 without significant changes in digester performance. When the relationship starts to increase, this is a warning that undesirable changes will occur unless the increase is stopped. If the relationship increases above 0.5, the composition of the gas produced can change very rapidly, followed by changes in the rate of gas production, and finally pH.

In a healthy and properly functioning digester, the processes or biological action taking place inside the digester are in equilibrium. When fresh sludge is pumped into a digester, some of the organisms in the digester convert this material to volatile (organic) acids. In a properly operated digester, other organisms feed on the newly produced volatile acids and eventually convert the acids to methane (CH_4) gas, which is burnable and carbon dioxide (CO_2). If too much raw sludge is pumped to the digester or the digester is not functioning properly, an excess of volatile acids are produced. If excessive amounts of volatile acids are produced, an acid environment unsuitable for some of the organisms in the digester will develop and the digester may cease to function properly unless the alkalinity increases too.

Routine volatile acids and alkalinity determinations during the start-up process for a new digester are a must in bringing the digester to a state of satisfactory digestion.

Routine volatile acids and alkalinity determinations during digestion are important in providing the information which will enable the operator to determine the health of the digester.

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[15] Thief Hole. A digester sampling well.

For digester control purposes, the volatile acid/alkalinity relationship should be determined. When the volatile acid/alkalinity relationship is from less than 0.1/1.0 to 0.5/1.0, the loading and seed retention of the digester are under control. When the relationship starts increasing and becomes greater than 0.5/1.0, the digester is out of control and will become "stuck" unless effective corrective action is taken.

B. What is Tested?

Sample	Desirable Range
Recirculated Sludge	150 - 600 mg/l (expect trouble if alkalinity less than two times volatile acids)

METHOD A

(Silic Acid Method)

C. Apparatus

1. Centrifuge or filtering apparatus.
2. Two 50 ml graduated cylinders.
3. Two medicine droppers.
4. Crucibles, Gooch or fritted glass
5. Filter flask
6. Vacuum source
7. One 50 ml beaker
8. Two 5 ml pipettes
9. Buret

D. Reagents

1. Silicic acid, solids, 100-mesh. Remove fines from solid portion of acid by slurrying the acid in distilled water and removing the supernatant after allowing settling for 15 minutes. Repeat the process several times. Dry the washed acid solids in an oven at 103°C and then store in a desiccator.

2. Chloroform-butanol reagent. Mix 300 ml chloroform, 100 ml n-butanol, and 80 ml 0.5 N H_2SO_4 in separatory funnel and allow the water and organic layers to separate. Drain off the lower organic layer through filter paper into a dry bottle.

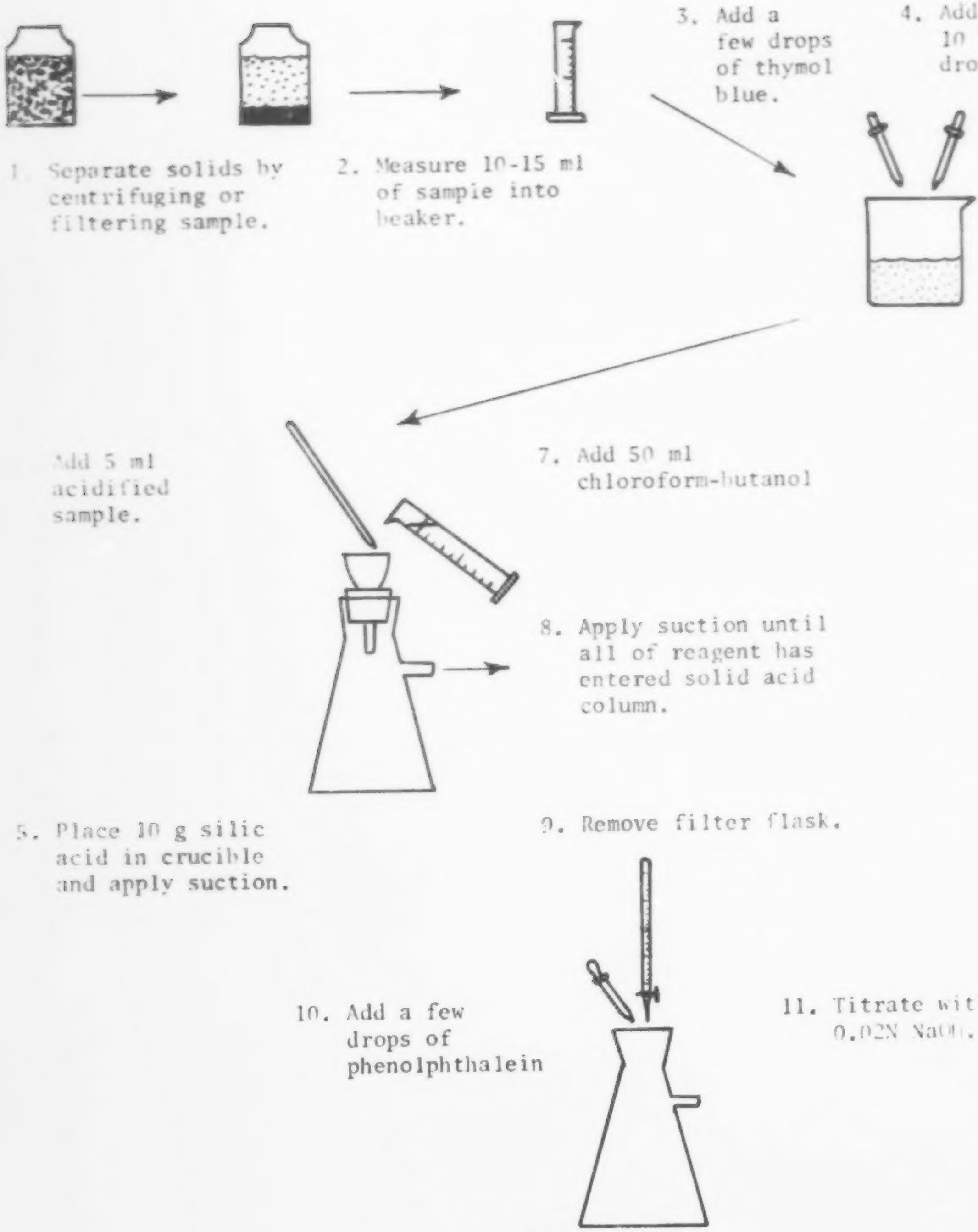
3. Thymol blue indicator solution. Dissolve 80 mg thymol blue in 100 ml absolute methanol.

4. Phenolphthalein indicator solution. Dissolve 80 mg phenolphthalein in 100 ml absolute methanol.

5. Sulfuric acid, 10 N.

6. Standard solium hydroxide reagent, 0.02 N. Prepare in absolute methanol from conc. NaOH stock solution in water.

E. Outline of Procedure



1. Separate solids by centrifuging or filtering sample.
2. Measure 10-15 ml of sample into beaker.
3. Add a few drops of thymol blue.
4. Add 10 N H_2SO_4 dropwise until thymol blue turns red

Add 5 ml acidified sample.

7. Add 50 ml chloroform-butanol
8. Apply suction until all of reagent has entered solid acid column.

5. Place 10 g silic acid in crucible and apply suction.

9. Remove filter flask.

10. Add a few drops of phenolphthalein
11. Titrate with 0.02N NaOH.

PROCEDURE

1. Centrifuge or filter enough sludge to obtain a sample of 10 to 15 ml. This same sample and filtrate should be used for both the volatile acids test and the total alkalinity test.

2. Measure volume (10 to 15 ml) of sample and place in a beaker.

3. Add a few drops of thymol blue indicator solution.

4. Add 10 N H_2SO_4, dropwise, until thymol blue color just turns to red.

5. Place 10 grams of silicic acid (solid acid) in crucible and apply suction. This will pack the acid material and the packed material is sometimes called a column.

6. With a pipette, distribute 5.0 ml acidified sample (from step 4) as uniformly as possible over the column. Apply suction briefly to draw the acidified sample into the silicic acid column. Release the vacuum as soon as the sample enters the column.

7. Quickly add 50 ml chloroform-butanol reagent to the column.

8. Apply suction and stop just before the last of the reagent enters the column.

9. Remove the filter flask from the crucible.

10. Add a few drops of phenolphthalein indicator solution to the liquid in the filter flask.

11. Titrate with 0.02 N NaOH titrant in absolute methanol, taking care to avoid aerating the sample. Nitrogen gas or CO_2 - free air delivered through a small glass tube may be used both to mix the sample and to prevent contact with atmospheric CO_2 during titration [CO_2 - free air may be obtained by passing air through ascarite or equivalent].

    Volume of NaOH used in sample titration, a = _______ ml.

12. Repeat the above procedure using a blank of distilled water.

    Volume of NaOH used in blank titration, b = ________ml.

F. Precautions

1. The sludge sample must be representative of the digester. The sample line should be allowed to run for a few minutes before the sample is taken. The sample temperature should be as warm as the digester itself.

2. The sample for the volatile acids test should not be taken immediately after charging the digester with raw sludge. Should this be done, the raw sludge may short-circuit to the withdrawal point and result in the withdrawal of raw sludge rather than digested sludge. Therefore, after the raw sludge has been fed into the tank, the tank should be well mixed by recirculation or other means before a sample is taken.

3. If a digester is performing well with low volatile acids and then if one sample should unexpectedly and suddenly give a high value, say over 1000 mg/l of volatile acids, do not become alarmed. The high result may be caused by a poor, nonrepresentative sample of raw sludge instead of digested sludge. Resample and retest. The second test may give a more typical value. When increasing volatile acids and decreasing alkalinity are observed, this is a definite warning of approaching control problems. Corrective action should be taken immediately, such as reducing the feed rate, reseeding from another digester, maintaining optimum temperatures, improving digester mixing, decreasing sludge withdrawal rate, or cleaning the tank of grit and scum.

G. Example

Equivalent Weight of Acetic Acid, A = 60 mg/ml

Volume of Sample, B = 10 ml

Normality of NaOH titrant, N = 0.02 N

Volume of NaOH used in sample titration, a = 2.3 ml

Volume of NaOH used in blank titration, b = 0.5 ml

H. Calculation

$$\text{Volatile Acids, mg/l (as acetic acid)} = \frac{A \times 1000 \text{ ml/l} \times N\,(a - b)}{B}$$

$$= \frac{60 \text{ mg/ml} \times 1000 \text{ ml/l} \times 0.02\,(2.3 \text{ ml} - 0.5 \text{ ml})}{10 \text{ ml}}$$

$$= 216 \text{ mg/l}$$

METHOD B

(Nonstandard Titration Method)

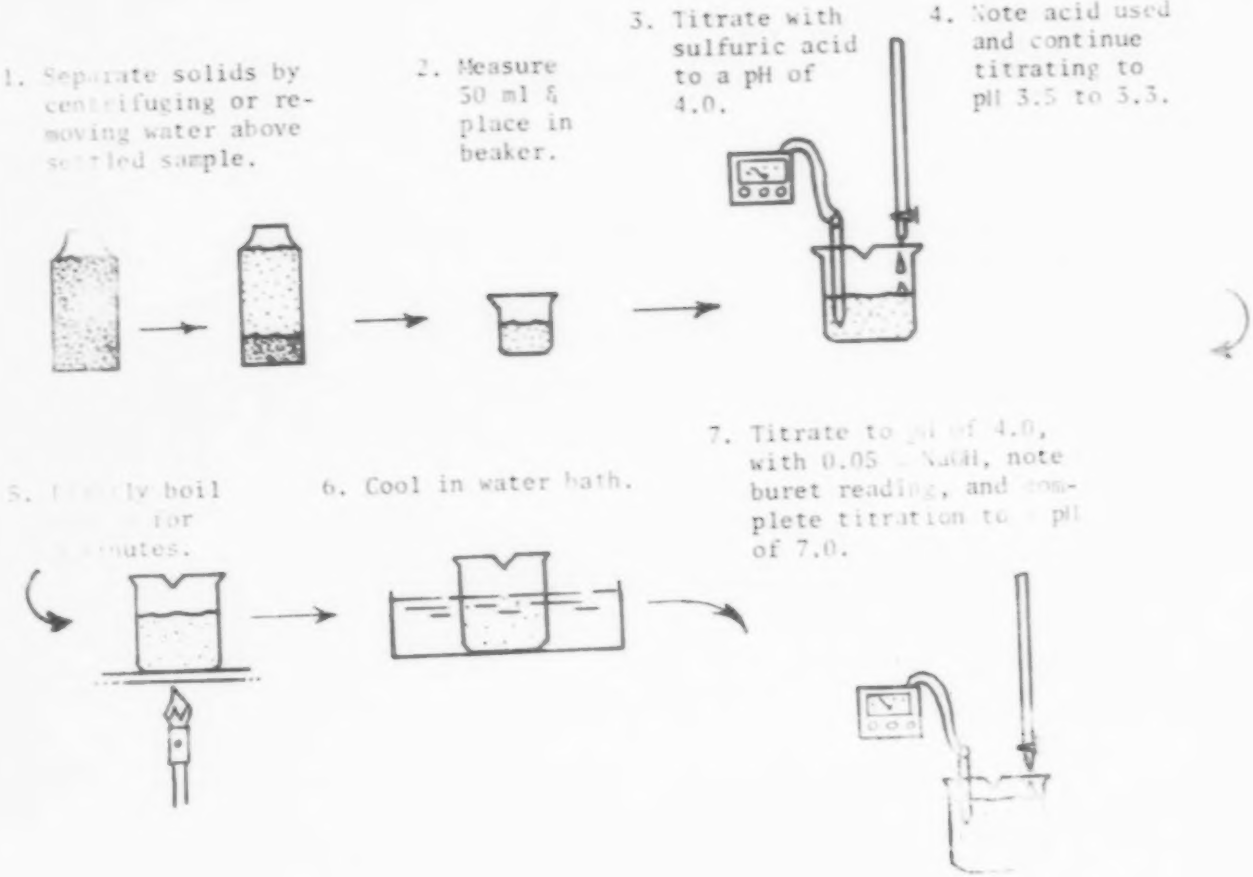
C. Apparatus

1. One pH meter.
2. One adjustable hot plate.
3. Two Burets and stand.
4. One 100 ml beaker.

D. Reagents

1. pH 7.0 buffer solution
2. pH 4.0 buffer solution
3. Standard acid.
4. Standard base.

E. Outline of Procedure



1. Separate solids by centrifuging or removing water above settled sample.

2. Measure 50 ml & place in beaker.

3. Titrate with sulfuric acid to a pH of 4.0.

4. Note acid used and continue titrating to pH 3.5 to 3.3.

5. Briefly boil [illegible] for [illegible] minutes.

6. Cool in water bath.

7. Titrate to pH of 4.0, with 0.05 [illegible] NaOH, note buret reading, and complete titration to [illegible] pH of 7.0.

PROCEDURE

1. Buffer the pH meter at 7.0 and check pH before treatment of sample to remove the solids. Filtration is not necessary. Decanting (removing water above settled material) or centrifuging sample is satisfactory. Do not add any coagulant aids.

2. Titrate 50 ml of the sample in a 100 ml beaker to pH 4.0 with the appropriate strength sulfuric acid (depends on alkalinity), note acid used, and continue to pH 3.5 to 3.3. A magnetic mixer is extremely useful for this titration.

3. Carefully buffer pH meter at 4.00 while lightly boiling the sample a minimum of three minutes. Cool in cold water bath to original temperature.

4. Titrate sample with standard 0.050 N sodium hydroxide up to pH 4.00, and note buret reading. Complete the titration at pH 7.0. (If this titration consistently takes more than 10 ml of the standard hydroxide, use 0.100 N NaOH.)

5. Calculate volatile acid alkalinity (alkalinity between pH 4.0 and 7.0).

$$\text{Volatile Acid Alkalinity} = \frac{\text{ml } 0.050 \text{ N NaOH} \times 2500}{\text{ml Sample}}$$

   For a 50 ml sample the volatile acid alkalinity equals 50 x ml 0.050 N NaOH, or 100 x ml 0.100 N NaOH.

6. Calculate volatile acids.

   Case 1: > 180 mg/1 volatile acid alkalinity.

   Volatile Acids = Volatile Acid Alkalinity x 1.50

   Case 2: < 180 mg/1 volatile acid alkalinity

   Volatile Acids = Volatile Acid Alkalinity x 1.0

   Steps 1 and 2 will give the analyst the pH and total alkalinity, two control tests normally run on digesters. The difference between the total and the volatile acid alkalinity is bicarbonate alkalinity. The time required for Steps 3 and 4 is about ten minutes.

   This is an acceptable method for digester control to determine the volatile acid/alkalinity relationship, but not of sufficient accuracy for research work.

For details regarding this test see DeLallo, R., and Albertson, G.E., *Volatile Acids by Direct Titration*, Water Pollution Control Federation, Vol. 33, No. 4, pp 356-365, April 1961. The procedure is reproduced from the article.

F. Example and Calculation

Titration of pH 4.0 to 7.0 of a 50 ml sample required 8 ml of 0.05 N NaOH.

Step 5 - Calculate volatile acid alkalinity (alkalinity between pH 4.0 and 7.0).

$$\text{Volatile Acid Alkalinity, mg/l} = \frac{\text{ml 0.05 N NaOH x 2500}}{\text{ml Sample}}$$

$$= \frac{\text{8 ml x 2500}}{\text{50 ml}}$$

$$= \text{400 mg/l}$$

Step 6 - Calculate volatile acids.

Case 1: 400 mg/l > [illegible] mg/l. Therefore,

$$\text{Volatile Acids, mg/l} = \text{Volatile Acid Alkalinity x 1.50}$$

$$= \text{400 mg/l x 1.50}$$

$$= \text{600 mg/l}$$

QUESTION

30.A What is the volatile acid concentration [illegible] digester if a 50 ml sample required [illegible] ml of 0.05 NaOH for a titration from a pH of 4.0 to 7.0

Total Alkalinity

A. Discussion

Tests for total alkalinity of digesters are normally run on settled supernatant samples. The alkalinity of the recirculated sludge is a measure of the buffer capacity in the digester. When organic matter in a digester is decomposed anaerobically, organic acids are formed which could lower the pH, if buffering materials (buffer capacity) were not present. If the pH drops too low, the organisms in the digester could become inactive or die and the digester becomes upset (no longer capable of decomposing organic matter).

For digester control purposes, the volatile acid/alkalinity relationship should be determined. When the volatile acid/alkalinity relationship is from less than 0.1/1.0 to 0.5/1.0, the loading and seed retention of the digester are under control. When the relationship starts increasing and becomes greater than 0.5/1.0, the digester is out of control and will become stuck unless effective corrective action is taken. The pH will not be out of range as long as the volatile acid/alkalinity relationship is low. This relationship gives a warning before trouble starts.

All samples must be settled so that a liquid free of solids is available for the test. Tests cannot be calculated correctly if solids are in the sample.

B. What is Tested?

Sample	Common [illegible]
Recirculated Sludge	2-10 Times [illegible]

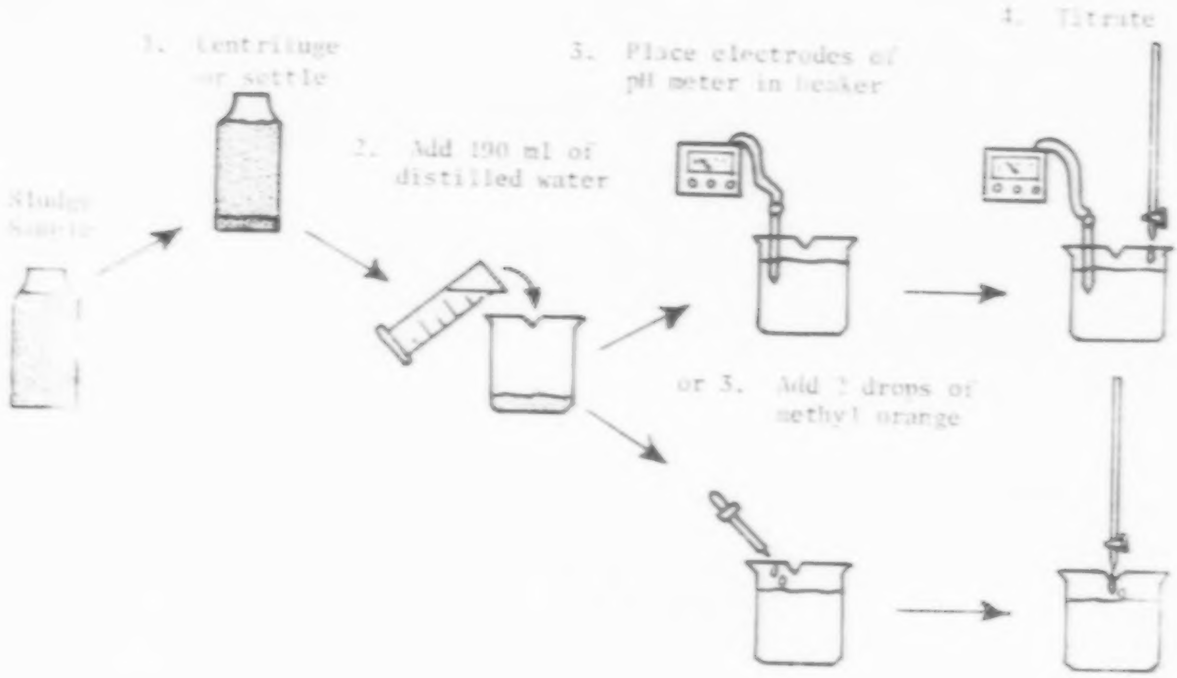
C. Apparatus

1. Centrifuge and centrifuge tubes, or settling cylinder.
2. Graduated cylinders (25 ml and 100 ml)
3. 50 ml Buret
4. 400 ml Erlenmeyer Flask or 400 ml beaker
5. pH Meter or a methyl orange chemical color indicator may be used (see Procedure)

D. Reagents

1. Sulfuric Acid, 0.2 N. Prepare stock solution of approximately 0.1 N by cautiously adding 2.8 ml of concentrated sulfuric acid (H_2SO_4) to 1 liter of distilled water. Dilute 200 ml of the 0.1 N stock solution to 1 liter with boiled distilled water. Standardize against 0.02 N sodium carbonate (Step 2).
2. Sodium Carbonate, 0.02 N. Dry in oven before weighing. Dissolve 1.06 g of anhydrous sodium carbonate (Na_2CO_3) in boiled distilled water and dilute to 1 liter with distilled water.
3. Methyl Orange Chemical Color Indicator. Dissolve 0.5 g methyl orange in 1 liter of distilled water.

E. Procedure


Sludge Sample
1. Centrifuge or settle
2. Add 190 ml of distilled water
3. Place electrodes of pH meter in beaker
4. Titrate
or 3. Add 2 drops of methyl orange

This procedure is followed to measure the alkalinity of a sample and also the alkalinity of a distilled water blank.

1. Take a clean 400 ml beaker and add 10 ml or less of clear supernatant (in case of water or distilled water, use 200 ml sample). Select a sample volume that will give a useable titration volume. If the liquid will not separate from the sludge by standing and a centrifuge is not available, use the top portion of the sample. This same sample and filtrate should be used for both the total alkalinity test and the volatile acids test.

2. Add 190 ml distilled water (in case of water or distilled water determination skip this step).

3. Place the electrodes of pH meter into the 400 ml beaker containing the sample.

4. Titrate to a pH of 4.5 with 0.02 N sulfuric acid. (In case of a lack of pH meter, add 2 drops of methyl orange indicator. In this case, titrate to the first permanent change of color to a red-orange color. Care must be exercised in determining the change of color and your ability to detect the change will improve with experience.)

5. The alkalinity of the distilled water should be checked and if significant, subtracted from the calculation.

6. Calculate alkalinity.

Alkalinity of Distilled Water, mg/l = ml of 0.02 N H_2SO_4 x 5*

Total Alkalinity, mg/l = ml of 0.02 N H_2SO_4 x 100* - mg/l alkalinity of distilled H_2O

F. Example

Results from alkalinity titrations on:

1. Distilled Water 4 ml 0.02 N H_2SO_4

2. Recirculated Sludge 1[illegible].8 ml 0.02 N H_2SO_4

G. Calculations

Alkalinity of Distilled H_2O, mg/l = ml of 0.02 N H_2SO_4 x 5

= 4 ml x 5

= 20 [illegible]

---

*Use 5 if measuring alkalinity of water or distilled water (200 ml sample) and 100 if measuring alkalinity of sludge (10 ml sample).

Total Alkalinity, mg/l, of recirculated sludge = ml of 0.02 N H_2SO_4 x 100 - mg/l alkalinity of distilled H_2O

= 19.8 ml x 100 - 20 mg/l

= 1980 mg/l - 20 mg/l

= 1960 mg/l

QUESTIONS

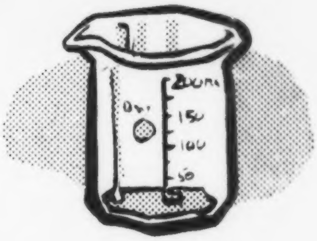
20.B Why would you run a total alkalinity test on recirculated sludge?

20.C What is meant by the buffer capacity in a digester?

20.D If the total alkalinity in a digester is 2000 mg/l and the volatile acids concentration is 300 mg/l per liter, what is the volatile acid/alkalinity relationship?

21. Volatile Solids

See Total Solids.


200 ml
150
100
50

END OF LESSON 8 OF 8 LESSONS

on

Laboratory Procedures and Chemistry

DISCUSSION AND REVIEW QUESTIONS

(Lesson 8 of 8 Lessons)

Chapter 14. Laboratory Procedures and Chemistry

Name ______________________________ Date ____________

Write the answers to these questions in your notebook. The problem numbering continues from Lesson 7.

32. Why are solids only weighed to the nearest 0.01 gram when determining the total and volatile solids content of digesters?

33. What is a thief hole?

34. What relationship is the critical control factor in digester operation?

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14.6 RECOMMENDED GENERAL LABORATORY SUPPLIES

Supplies needed in addition to apparatus listed for tests. Source: WPCF Publication No. 18, *Simplified Laboratory Procedures for Wastewater Examination.*

Quantity	Description
12	Pinch clamps, medium
200	Corks, assorted
1	Cork borer set, sizes 1 through 6
1	Cork borer sharpener
2 lb	Glass tubing, 8 mm
4	Thermometers, -20° to 100°C
40 ft	Rubber tubing, 1/4-in. ID, 3/32-in. wall
2 lb	Rubber stoppers, assorted (sizes 6 through 12)
1	Tripod, concentric ring, 6 in. OD
1	Latest edition, *Standard Methods for the Examination of Water & Wastewater*
2	Funnels, 60 mm
2	Funnels, 100 mm
2 pair	Balance watch glasses, 3 in.
4	Beakers, Pyrex, 1000 ml
4	Beakers, Pyrex, 600 ml
6	Beakers, Pyrex, [illegible]
[illegible]	Beakers, Pyrex, 250 ml
[illegible]	Beakers, Pyrex, 100 ml
4	Beakers, Pyrex, [illegible] ml
2	Bunsen burners
2	Brushes, medium
2	Brush, [illegible]
2	Brush, [illegible]
2	Brush, flask
2	Aprons, plastic, [illegible] in. length
3	Wire gauzes, 4 x 4 in.
3	Triangles, 2-1/2 in. per side
1 tube	Stopcock lubricant

SUPPLEMENTAL EQUIPMENT FOR THE BOD TEST

Quantity	Description
12	Flask, Erlenmeyer, 500 ml
12	Flask, Erlenmeyer, 250 ml
2	Pipettes, volumetric, 25 ml
2	Pipettes, volumetric, 10 ml
2	Pipettes, volumetric, 5 ml
2	Flasks, volumetric, graduated to contain and deliver 1000 ml
2	Flasks, volumetric, graduated to contain and deliver 500 ml
2	Flasks, volumetric, graduated to contain and deliver 100 ml
6	Bottles, 32 oz
6	Bottles, 16 oz
6	Bottles, 8 oz
24	BOD bottles, with funnel opening
2	Burets, 50 ml
1	Buret clamp, double
2	Bottles, dropping, 30 ml
2	Spatulas, 75-mm blade
3	Bottles, storage, 2-1/2 gal
1	Buret support, medium
9 lb	Sulfuric acid, CP
5 lb	Sodium hydroxide pellets, CP
12	Bulb, rubber, pipette, 2 ml
24	Holder, rubber, stopper
4	Flask, volumetric, w/o stopper, 100 ml
2 lb	Potassium iodide, CP
1 lb	Starch, soluble potato
1 lb	Sodium thiosulfate, CP
5 lb	Manganous sulfate, CP
100 g	Sodium azide, CP
1 lb	Magnesium sulfate
1/4 lb	Ferric chloride
1 lb	Potassium phosphate, mono-basic
1 lb	Potassium phosphate, dibasic

Quantity	Description
1 lb	Sodium phosphate, dibasic heptahydrate
1/4 lb	Ammonium chloride
1 oz	Potassium bi-iodate, primary standard
1 lb	Potassium dichromate
10 g	Sodium diethyldithio carbamate
1	Incubator, BOD
1	Refrigerator
1 lb	Calcium chloride, 20 mesh

SUPPLEMENTAL EQUIPMENT FOR THE CHLORINE RESIDUAL TEST

Quantity	Description
1	Comparator, water analysis
1	Disc for comparator, chlorine
6 lb	Hydrochloride acid, CP
25 g	Orthotolidine dihydrochloride

SUPPLEMENTAL EQUIPMENT FOR SOLIDS ANALYSIS

Quantity	Description
1	Brush, camel hair, 1-in. wide
1	Balance with cover
1	Weights, balance set, 50 g
12	Crucibles, Gooch, No. 4
2	Holders, crucible
2	Cylinder, graduated, 1000 ml
2	Cylinder, graduated, 500 ml
2	Cylinder, graduated, 250 ml
4	Cylinder, graduated, 100 ml
4	Cylinder, graduated, 50 ml
2	Cylinder, graduated, 25 ml
1	Cylinder, graduated, 10 ml
1	Desiccator, 250 mm
1	Desiccator plate
12	Dishes, evaporating, size 0

Quantity	Description
3	Flask, filtering, 500 ml
2	Pipettes, 25 ml
6	Pipettes, 10 ml
2	Pipettes, 5 ml
1	Hot plate, 660 w
2	Tongs, crucible
1	Tongs, furnace, 18 in.
8 ft	Tubing, rubber, (heavy) 1/4-in. ID
2	Filter pumps
1	Clock, interval timer, 2 hr
1	Furnace, muffle
2 boxes	Paper, filter, glass fiber, 2.4 cm
1	Water baths, four-hole
1	Balance, platform, triple beam
2	Bottles, washing, polyethylene, 500 ml
6	Pencils, wax, red
2 boxes	Filter paper, 12.5 cm, Whatman No. 41
1 bottle	Ink, marking, black
1 lb	Rod, glass, 6 mm
1	File, triangular, 4 in.
12	Bulb, rubber, pipet, 2 oz
1	Balance desiccator
1	Oven, drying
24	2.4 cm glass fiber filter
2	Buchner funnel, size 2A
6	Tube "T", connecting, 1/4-in.
5 lb	Drierite

SUPPLEMENTAL EQUIPMENT FOR COLIFORM GROUP BACTERIA ANALYSES

Quantity	Description
1	Sterilizer or autoclave
12	3 mm wire transfer loop
24	Pipets, measuring, 10 ml
48	Pipets, measuring, 1 ml, or quantity of disposable sterile pipets

Many equipment suppliers will furnish suggested equipment lists upon request and indication of size of plant and tests being performed. Lists may be obtained from:

Central Scientific Company 1700 Irving Park Road Chicago, Illinios	Van Waters & Rogers Post Office Box 2062 Terminal Annex Los Angeles, California 90054

14.7 ADDITIONAL READING

a. MOP 11

b. New York Manual, pages 127-148

c. Texas Manual, pages 565-587

d. *Laboratory Procedures for Operators of Water Pollution Control Plants*, Nagano, Joe. Obtain from Secretary-Treasurer, California Water Pollution Control Association, P.O. Box 61, Lemon Grove, California 92045. Price $3.25 to members of the CWPCA; $4.25 to others.

e. *Simplified Laboratory Procedures for Wastewater Examination*, WPCF Publication No. 18, Water Pollution Control Federation, 3900 Wisconsin Avenue, Washington, D.C. 20016. Price $2.00 to members; $4.00 to others. Indicate your member association when ordering.

f. *Standard Methods for Examination of Water and Wastewater*, produced by APHA, AWWA, and WPCF, Water Pollution Control Federation, 3900 Wisconsin Avenue, Washington, D.C. 20016. Price $16.50 to members prepaid only; otherwise $22.50 plus postage. Indicate your member association when ordering.

g. *Chemistry for Sanitary Engineers*, Sawyer, Clair N. and McCarty, Perry L., McGraw-Hill Book Company, New York, 1967. Price $13.50.

h. *Methods for Chemical Analysis of Water and Wastes*, 1971, Environmental Protection Agency, Water Quality Office, Analytical Quality Control Laboratory, 1014 Broadway, Cincinnati, Ohio 45202. For sale by Superintendent of Documents, Government Printing Office, Washington, D.C. 20402, Stock Number 5501-0667. Price $3.00.

SUGGESTED ANSWERS

Chapter 14. Laboratory Procedures and Chemistry

14.2A A bulb should always be used to pipette wastewater or polluted water to prevent infectious materials from entering your mouth.

14.2B Inoculations are recommended to reduce the possibility of contracting diseases.

14.2C Immediately wash area where acid spilled with water and neutralize the acid with sodium carbonate or bicarbonate.

14.2D True. You may add acid to water, but never reverse.

14.2E Work clothes should be changed before going home at night to prevent carrying unsanitary materials and diseases home which could infect you and your family.

14.3A The largest sources of errors found in laboratory results are usually caused by improper sampling; poor preservation; and lack of sufficient mixing, compositing, and testing.

14.3B A representative sample must be collected or the test results will not have any significant meaning. To efficiently operate a wastewater treatment plant, the operator must rely on test results to indicate to him what is happening.

14.3C A proportional composite sample may be prepared by collecting a sample every hour. The size of this sample is proportional to the flow when the sample is collected. All of these proportional samples are mixed together to produce a proportional composite sample. If an equal volume of sample was collected each hour and mixed, this would be simply a composite sample.

3.A The dangers encountered in running the CO_2 on digester gas include:

1. Digester gas contains methane, which is explosive when mixed with air.
2. The CO_2 gas absorbent is harmful to your skin.

3.B $$\% \ CO_2 = \frac{(\text{Total Volume, ml} - \text{Gas Remaining, ml}) \times 100\%}{\text{Total Volume, ml}}$$

$$= \frac{(128 \text{ ml} - 73 \text{ ml}) \times 100\%}{128 \text{ ml}}$$

$$\begin{array}{r} 128 \\ -\ 73 \\ \hline 55 \end{array}$$

$$= \frac{55}{128} \times 100\%$$

$$= 43\%$$

$$\begin{array}{r} .43 \\ 128/\overline{55.0} \\ \underline{51\ 2} \\ 3\ 80 \\ 3\ 84 \end{array}$$

4.A The COD test is a measure of the strength of a waste in terms of its chemical oxygen demand. It is a good estimate of the first-stage oxygen demand. (Either answer is acceptable.)

4.B The advantage of the COD test over the BOD test is that you don't have to wait five days for the results.

5.A Plant effluents should be chlorinated for disinfection purposes to protect the bacteriological quality of the receiving waters.

5.B The idometric method gives good results with samples containing wastewater, such as plant effluent or receiving waters. Orthotolidine will give satisfactory results if used within 20 minutes of the application of chlorine; however, the entire chlorine demand may not yet have been satisfied. Amperometric titration gives satisfactory results, but the equipment is expensive.

6.A The clarity test indicates the relative change of depth you can see down in the final clarifier or contact basin. This reflects a visual comparison of color, solids, and turbidity from one test to the next. OR Indication of quality of effluent.

6.B When clarity is measured under different conditions the results can not be compared. You won't be able to tell whether your plant performance is improving, staying the same, or deteriorating.

7.A Sodium thiosulfate crystals should be added to sample bottles for coliform bacteria tests before sterilization to neutralize any chlorine that may be present when the sample is collected. Care must be taken not to wash the bottles out when a sample is collected.

7.B 121°C within 15 minutes.

7.C

Dilutions	-2	-3	-4	-5
Readings	5	1	2	0

MPN = 63,000/100 ml

7.D The number of coliforms is estimated by counting the number of colonies grown on the membrane filter.

8.A
$$\text{DO Saturation, \%} = \frac{\text{DO of Sample, mg/l} \times 100\%}{\text{DO at Saturation, mg/l}}$$

$$= \frac{(7.9\text{ mg/l})\ 100\%}{11.3\text{ mg/l}}$$

$$= 70\%$$

$$\begin{array}{r} .699 \\ 11.3\,/\overline{\,7.9\,0} \\ \underline{6\ 7\ 8} \\ 1\ 1\ 20 \\ \underline{1\ 0\ 17} \\ 1\ 030 \\ 1\ 017 \end{array}$$

8.B To calibrate the DO probe in an aeration tank, a sample of effluent can be collected and split. The DO of the effluent is measured by the modified Winkler procedure, and the probe DO reading is adjusted to agree with the Winkler results.

8.C When the DO in the aeration tank is very low, the copper sulfate-sulfamic acid procedure can give high results. The results are high because oxygen enters the sample from the air when the sample is collected, when the copper sulfate-sulfamic acid inhibitor is added, while the solids are settling, and when the sample is transferred to a BOD bottle for the DO test.

8.D BOD test or volatile solids test.

8.E To prepare dilutions for a cannery waste with an expected BOD of 2000 mg/l, take 10 ml of sample and add 90 ml of dilution water to obtain a new sample with an estimated BOD of 200 mg/l (10 to 1 dilution):

$$\text{BOD Dilution, ml} = \frac{1200}{\text{Estimated BOD, mg/l}}$$

$$= \frac{1200}{200}$$

$$= 6 \text{ ml}$$

8.F

$$\text{BOD, mg/l} = \left(\text{Initial DO of Diluted Sample, mg/l} - \text{DO of Diluted Sample After 5-Day Incubation, mg/l}\right)\left(\frac{\text{BOD Bottle Vol., ml}}{\text{Sample Volume, ml}}\right)$$

$$= (7.5 \text{ mg/l} - 3.9 \text{ mg/l})\left(\frac{300 \text{ ml}}{2 \text{ ml}}\right)$$

$$= (3.6 \text{ mg/l})\ (150)$$

$$= 540 \text{ mg/l}$$

8.G Samples for the BOD test should be collected before chlorination because chlorine interferes with the organisms in the test. It is difficult to obtain accurate results with dechlorinated samples.

8.H A solution of sodium thiosulfate at 0.0375 N is very weak and unstable and will not remain accurate over two weeks.

9.A (1) You would measure the H_2S in the wastewater to know the strength of H_2S and an indication of the corrosion taking place on the concrete.

(2) H_2S in the atmosphere produces a rotten egg odor. It is indicative of anaerobic decomposition of organics in wastewater which occurs in the absence of oxygen.

10.A (1) To measure plant influent pH with a paper tape, collect representative sample, mix sample with a clean stirring rod, and dip tape in sample while it is still moving. Compare tape color with package color and record results.

(2) To measure raw sludge pH with a paper tape first allow raw sludge sample to settle. Dip tape in liquid at top, compare resulting color, and record results.

pH of both samples should be measured in place or as soon as possible.

10.B Precautions to be exercised when using a pH meter include:

(1) Prepare fresh buffer solution weekly for calibration purposes.

(2) pH meter, samples, and buffer solutions should all be at the same temperature.

(3) Watch for erratic results arising from faulty operation of pH meter or fouling of electrodes with interfering matter.

11.A Settleability tests should be run on the mixed liquor to determine the settling characteristics of the sludge floc at regular intervals for 60 minutes. The results are used in the SVI and SDI determinations.

11.B The SVI is the volume in ml occupied by one gram of mixed liquor suspended solids after 30 minutes of settling.

11.C The SVI test is used to indicate changes in sludge characteristics.

11.D Sludge Density Index (SDI) = 100/SVI

12.A Sludge to Digester, gpd

$$= (\text{Total Set Sol Removed, ml/l})\ (1000)\ (\text{Flow, MGD})$$

$$= (10\ \text{ml/l} - 0.4\ \text{ml/l})\ (1000\ \text{mg/ml})\ (1\ \text{M Gal/day})$$

$$= \left(\frac{9.6\ \text{ml}}{\text{M mg}}\right)\left(\frac{1000\ \text{mg}}{\text{ml}}\right)\left(\frac{1\ \text{M Gal}}{\text{day}}\right)$$

$$= 9600\ \text{gpd}$$

This value may be reduced by 30 to 75% due to compaction of the sludge in the clarifier.

13.A The sludge age of a 200,000 gallon aeration tank that has 2000 mg/l mixed liquor suspended solids, primary effluent of 115 mg/l SS, and an average flow of 1.8 MGD:

Sludge Age, days

$$= \frac{\underset{.2\ \text{MG}}{\text{Vol of Aeration Tank}} \times \text{Sus Solids, 2000 mg/l}}{\text{Flow, MGD, 1.8} \times \text{Primary Effl, 115 mg/l}}$$

$$= \frac{0.2\ \text{MG} \times 2000\ \text{mg/l}}{1.8\ \text{MGD} \times 115\ \text{mg/l}}$$

$$= 1.93$$

14.A (1) Results from the graduated cylinder are available immediately, but different operators may interpret the results differently.

(2) Results are not available until the next day, but different operators will record the same result.

15.A If the supernatant solids test is greater than 5%, the supernatant could be placing a heavy solids load on the plant and the appropriate operational adjustments should be made.

16.A The specific gravity is very near that of H_2O and is not light enough to float nor heavy enough to settle.

16.B Solids calculations will be shown in detail here to illustrate the computational approach and the units involved. After you understand this approach, use of the laboratory work sheet on the following pages is more convenient.

a. Total Suspended Solids

Volume of Sample, ml = 100 ml
Weight of Dried Sample & Dish, grams = 19.3902 g
Weight of Dish (Tare Weight), grams = 19.3241 g
Dry Weight = 0.0661 g
or = 66.1 mg

$$\text{Total Suspended Solids, mg/l} = \frac{\text{Weight of Solids, mg} \times 1000 \text{ ml/l}}{\text{Volume of Sample, ml}}$$

$$= \frac{66.1 \text{ mg} \times 1000 \text{ ml/l}}{100 \text{ ml}}$$

$$= 661 \text{ mg/l}$$

b. Volatile Suspended Solids

Weight of Dried Sample & Dish, grams = 19.3902 g
Weight of Ash & Dish, grams = 19.3469 g
Weight Volatile, grams = 0.0433 g
or = 43.3 mg

$$\text{Volatile Suspended Solids, mg/l} = \frac{\text{Weight of Volatile, mg x 1000 ml}}{\text{Volume of Sample, ml}}$$

$$= \frac{(43.3\ \text{mg})\ (1000\ \text{ml/l})}{100\ \text{ml}}$$

$$= 433\ \text{mg/l}$$

c. Percent Volatile Solids

$$\%\ \text{Volatile Solids} = \frac{\text{Weight Volatile, mg x 100\%}}{\text{Weight Dry, mg}}$$

$$= \frac{433\ \text{mg}}{661\ \text{mg}} \times 100\%$$

$$= 65.5\%$$

$$\begin{array}{r} .655 \\ 661\overline{)433.0} \\ \underline{396\ 6} \\ 36\ 40 \\ \underline{33\ 05} \\ 3\ 350 \\ 3\ 305 \end{array}$$

d. Fixed Solids

Total Suspended Solids, mg/l = 661 mg/l

Volatile Suspended Solids, mg/l = 433 mg/l

Fixed Solids, mg/l = 228 mg/l

e. Percent Fixed Solids

Total Solids, % = 100.00%

Volatile Solids, % = 65.50%

Fixed Solids, % = 34.5 %

or

$$\%\ \text{Fixed} = \frac{\text{Fixed, mg}}{\text{Total, mg}} \times 100\%$$

$$= \frac{228\ \text{mg}}{661\ \text{mg}} \times 100\%$$

$$= 34.5\%\ \text{(Check)}$$

16.C Calculate Percent Reduction through Primary:

$$\% \text{ Removal} = \frac{(\text{In} - \text{Out})}{\text{In}} \times 100\%$$

In = Influent to plant or unit

Out = What is leaving plant or unit

$$= \frac{(221 \text{ mg/l} - 159 \text{ mg/l})}{221 \text{ mg/l}} \times 100\%$$

$$= \frac{62}{221} \times 100\%$$

$$= 28\% \text{ reduction through primary}$$

```
       .28
221 ⟌ 62.0
      44 2
      17 80
      17 68
```

Calculate Percent Removal by Secondary System:

$$\% \text{ Removal} = \frac{(\text{In} - \text{Out})}{\text{In}} \times 100\%$$

In = 159 mg/l SS in primary effluent

Out = 33 mg/l SS in final effluent

$$= \frac{(159 \text{ mg/l} - 33 \text{ mg/l})}{159 \text{ mg/l}} \times 100\%$$

$$= 79\% \text{ removal from primary effluent to final effluent}$$

```
        .79
159 ⟌ 126.0
      111 3
       14 70
       14 31
```

Calculate Overall Plant Efficiency:

$$\% \text{ Removal} = \frac{(\text{In} - \text{Out})}{\text{In}} \times 100\%$$

In = 221 mg/l SS in plant influent

Out = 33 mg/l SS in plant effluent

$$= \frac{(221 \text{ mg/l} - 33 \text{ mg/l})}{221 \text{ mg/l}} \times 100\%$$

$$= \frac{188}{221} \times 100\%$$

$$= 85.5\% \text{ overall plant removal}$$

16.D Calculate the pounds of solids removed per day by each unit:

Amount Removed, lb/day = Conc. Reduction, mg/l x Flow, MGD x 8.34 lb/gal

where MGD = million gallons per day

A. Influent, mg/l = 221 mg/l

Primary Effluent, mg/l = 159 mg/l

Primary Removal, mg/l = 62 mg/l

Amount Removed, lb/day (Primary) = (62 mg/l) (1.5 MGD) (8.34 lb/gal)

= 775.6 lbs/day removed by primary

B. Primary Effluent, mg/l = 159 mg/l

Final Effluent, mg/l = 33 mg/l

Secondary Removal, mg/l = 126 mg/l

Amount Removed, lb/day (Secondary) = (126 mg/l) (1.5 MGD) (8.34 lb/gal)

= 1576 lb/day removed by secondary

C. Influent, mg/l = 221 mg/l

Final Effluent, mg/l = 33 mg/l

Overall Removal, mg/l = 188 mg/l

Amount Removed, mg/l = (188 mg/l) (1.5 MGD) (8.34 lb/gal)

= 2351 lbs/day removed by plant

or = Primary Removal, lb/day + Secondary, lb/day

= 775 + 1576

= 2351 (Check)

16.E The advantages of the centrifuge over the regular suspended solids test are:

(1) Speed of answer! Not as accurate as other methods, but results are sufficiently close.

(2) Answers very acceptable if suspended solids concentration is below 1000 mg/l.

Disadvantage: Small plants cannot always afford the $500 or more cost of the centrifuge.

17.A Changes in influent temperature could indicate a new influent source. A drop in temperature could be caused by cold water from infiltration, and an increase in temperature could be caused by an industrial waste discharge.

17.B The thermometer should remain immersed in the liquid while being read for accurate results. When removed from the liquid, the reading will change.

17.C All thermometers should be calibrated against an accurate National Bureau of Standards thermometer because some thermometers can be purchased that are substantially inaccurate (off as much as 6°).

18.A Volatile solids found in a digester are organic compounds of either plant or animal origin.

18.B Volatile solids in a treatment plant represent the waste material that may be treated by biological processes.

20.A

$$\text{Volatile Acid Alkalinity, mg/l} = \frac{\text{ml } 0.05 \text{ N NaOH} \times 2500}{\text{ml Sample}}$$

$$= \frac{5 \text{ ml} \times 2500}{50 \text{ ml}}$$

$$= 250 \text{ mg/l}$$

Since 250 mg/l > 180 mg/l,

$$\text{Volatile Acids, mg/l} = \text{Volatile Acid Alkalinity} \times 1.50$$

$$= 250 \text{ mg/l} \times 1.50$$

$$= 375 \text{ mg/l}$$

20.B The alkalinity test is run to determine the buffer capacity and the volatile acids/alkalinity relationship in a digester.

20.C The buffer capacity in a digester as measured by the total alkalinity tests indicates the capacity of the digester to resist changes in pH.

20.D $$\frac{\text{Volatile Acid}}{\text{Alkalinity}} = \frac{300 \text{ mg/l}}{2000 \text{ mg/l}}$$

$$= 0.15$$

OBJECTIVE TEST

Chapter 14. Laboratory Procedures and Chemistry

Name ______________________________ Date ____________

Please write your name and mark the correct answers on the IBM answer sheet. There may be more than one correct answer to each question.

TRUE OR FALSE (1-10):

1. A rubber bulb should be used to pipette wastewater or polluted water.

    1. True
    2. False

2. Acid may be added to water, but not the reverse.

    1. True
    2. False

3. Always wear safety goggles when conducting any experiment in which there may be danger to the eyes.

    1. True
    2. False

4. Smoking and eating should be avoided when working with infectious material such as wastewater and sludge.

    1. True
    2. False

5. In the washing of hands after working with wastewater, the kind of soap is less important than the thorough use of soap.

    1. True
    2. False

6. The pH scale may range from 0 to 14, with 7 being a neutral solution.

    1. True
    2. False

7. If at all possible, samples for the BOD test should be collected before chlorination.

   1. True
   2. False

8. The COD test is a measure of the chemical oxygen demand of wastewater.

   1. True
   2. False

9. The BOD test is a measure of the organic content of wastewater.

   1. True
   2. False

10. The answers from the total solids and suspended solids test are always the same.

   1. True
   2. False

Possible definitions of the words listed below are given on the right. For each word listed on the left, try to find its definition on the right. Mark the number of the definition in the answer column for each word. For example, if the definition of a word is after the number 2, mark column 2 on your answer sheet after the word.

Word	Definition
	1. Surrounding
11. Aliquot	2. Capacity to resist pH change
12. Ambient	3. Portion of a sample
13. Blank	4. Inside
14. Buffer	5. Test run without sample

15. Large errors in laboratory tests may be caused by:

   1. Improper sampling
   2. Large samples
   3. Poor preservation
   4. Poor quality effluent
   5. Lack of mixing during compositing

16. The most critical factor in controlling digester operation is the:

    1. CO_2
    2. Gas production
    3. Volatile solids
    4. Volatile acids/alkalinity relationship
    5. pH

17. The COD test:

    1. Measures the biochemical oxygen demand
    2. Estimates the first-stage oxygen demand
    3. Measures the carbon oxygen demand
    4. Estimates the total oxygen demand
    5. Provides results quicker than the BOD test

18. A clarity test on plant effluent:

    1. Tells if the effluent is safe to drink
    2. Is measured by an amperometer
    3. Should always be measured at the same time
    4. Should always be measured under the same light conditions
    5. Is measured by a Secchi Disc

19. Coliform group bacteria are:

    1. Measured by the membrane filter method
    2. Measured by the multiple fermentation technique
    3. Measured by the modified Winkler procedure
    4. Harmful to humans
    5. Indicative of the potential presence of bacteria originating in the intestines of warm-blooded animals

20. The saturation concentration of dissolved oxygen in water does not vary with temperature.

    1. True
    2. False

21. DO probes are commonly used to measure dissolved oxygen in water in:

    1. Aeration tanks
    2. Sludge digesters
    3. Manholes
    4. Streams
    5. BOD bottles

22. Hydrogen sulfide:

    1. Reacts with moisture and oxygen to form a substance corrosive to concrete
    2. Is sometimes written as H_2S
    3. Smells like rotten eggs
    4. Is formed under aerobic conditions
    5. Should not be controlled in the collection system.

23. Results from the settleability test of activated sludge solids may be used to:

    1. Calculate SVI
    2. Calculate SDI
    3. Calculate sludge age
    4. Determine ability of solids to separate from liquid in final clarifier
    5. Calculate mixed liquor suspended solids.

24. Results of the settleable solids test run using Imhoff cones may be used to:

    1. Calculate the Imhoff Settling Index
    2. Calculate the efficiency of a treatment process.
    3. Calculate the pounds of solids pumped to the digester
    4. Indicate the quality of the influent
    5. Indicate the quality of the effluent

25. Precautions that must be observed in running the suspended solids-Gooch crucible test include:

    1. Collecting and testing a representative sample
    2. Proper temperature level in oven at all times
    3. Lack of leaks around and through the glass fiber
    4. Thoroughly mixing sample before testing
    5. Discarding any large chunks of material in sample

26. A chlorine residual should be maintained in a plant effluent:

    1. To keep the chlorinator working
    2. For disinfection purposes
    3. For testing purposes
    4. To protect the bacteriological quality of the receiving waters
    5. None of these

PLANT ______________________________

DATE ______________________________

SUSPENDED SOLIDS & DISSOLVED SOLIDS

SAMPLE							
Crucible							
Ml Sample							
Wt Dry & Dish Wt Dish							
Wt Dry							
$\text{mg/l} = \frac{\text{Wt Dry, gm} \times 1{,}000{,}000}{\text{Ml Sample}}$							
Wt Dish & Dry Wt Dish & Ash							
Wt Volatile							
$\% \text{ Vol} = \frac{\text{Wt Vol}}{\text{Wt Dry}} \times 100$							

BOD

Blank ______________

SAMPLE										
DO Sample										
Bottle #										
% Sample										
Blank or adj blank DO after incubation										
Depletion, 5 days										
Dep %										

Nitrate NO_3

Sample ______________ ______________

Graph Reading ______________ ______________

Sett. Solids

Sample ______________ ______________

Direct Ml/l ______________ ______________

COD

Sample ______________ ______________ ______________

Blank Titration ______________ ______________ ______________

Sample Titration ______________ ______________ ______________

Depletion ______________ ______________ ______________

$$\text{mg/l} = \frac{\text{Dep} \times N \text{ FAS} \times 8000}{\text{Ml Sample}}$$ ______________ ______________ ______________

Typical Laboratory Work Sheet

TOTAL SOLIDS

SAMPLE						
Dish No.						
Wt Dish & Wet Wt Dish						
Wt Wet						
Wt Dish + Dry Wt Dish						
Wt Dry						
% Solids $\frac{\text{Wt Dry}}{\text{Wt Wet}} \times 100\%$						
Wt Dish + Dry Wt Dish + Ash						
Wt Volatile						
% Volatile = $\frac{\text{Wt Vol}}{\text{Wt Dry}} \times 100\%$						
pH						
Vol. Acid						
Alkalinity as $CaCO_3$						

Grease (Soxlet) __________

Sample __________

Ml Sample __________

Wt Flask + Grease
Wt Flask __________

Wt Grease __________

mg/l = $\frac{\text{Wt Grease, mg} \times 1000}{\text{Ml Sample}}$ __________

H_2S (Gas) (Starch-Iodine)

Blank __________ Ml
Sample __________ Ml
Diff __________ Ml
Diff x .68 __________ mg/l
mg/l x 43.6 __________ grain/100 cu ft

Typical Laboratory Work Sheet (Continued)

OPERATION OF WASTEWATER TREATMENT PLANTS

A Field Study Training Program

prepared by

Sacramento State College
Department of Civil Engineering

in cooperation with the

California Water Pollution Control Association

****

Kenneth D. Kerri, Project Director
Bill B. Dendy, Co-Director
John Brady, Consultant
William Crooks, Consultant

****

for the

Environmental Protection Agency
Water Quality Office
Technical Training Grant No. 5TT1-WP-16-03

1970